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**PROCEEDINGS OF THE
2002 SUGAR PROCESSING
RESEARCH CONFERENCE**

**Advances in the Chemistry and Processing
of Beet and Cane Sugar**



**MARCH 10-13, 2002
NEW ORLEANS, LOUISIANA**

**Sponsored by
Sugar Processing Research Institute, Inc.
New Orleans, Louisiana**

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PREFACE

The 2002 Sugar Processing Research Conference is one in a series of Conferences held in alternate years to provide a forum for the exchange of information among technical leaders of the sugar industry and to report on new and noteworthy developments. The Conference was sponsored by Sugar Processing Research Institute, Inc. (SPRI).

The program for this Conference was arranged by Mary An Godshall. The Conference Coordinators were Shirley Saucier and Xavier Miranda. These Proceedings were edited by Mary An Godshall.

Sugar Processing Research Institute, Inc., acknowledges the contribution in kind to the support of this conference by the Southern Regional Research Center, Agriculture Research Service, United States Department of Agriculture. We also gratefully acknowledge the support of The American Sugar Refining Company, who provided a tour of its Domino - Chalmette Refinery and lunch on the grounds for the delegates.

The series, Proceedings of the Sugar Processing Research Conference, of which this is the eleventh issue, continues the Proceedings of the Technical Sessions on Cane Sugar Refining Research, which was published every other year from 1964 to 1980. For individual copies of this volume, as well as back issues of the former series as long as the supply lasts, write to Sugar Processing Research Institute, Inc., 1100 Robert E. Lee Blvd., New Orleans, Louisiana 70124. Before 1986, Proceedings were published by the Agriculture Research Service, U.S. Department of Agriculture. Since 1988, Proceedings have been published by Sugar Processing Research Institute, Inc.

Mary An Godshall
Managing Director
Sugar Processing Research Institute, Inc.

Dr. Charley Richard, President
Sugar Processing Research Institute, Inc.

SUGAR PROCESSING RESEARCH INSTITUTE, INC.

Sugar Processing Research Institute, Inc., is an independent, non-profit research institute supported by the international sugarcane and sugarbeet production and refining industries and their supplier and user companies. The Institute is housed at the Southern Regional Research Center of the United States Department of Agriculture, Agricultural Research Service, under a Memorandum of Understanding with USDA. The association with USDA offers many synergies for the benefit of the sugar industry. The SPRI organization is unique in that it undertakes both beet and cane sugar processing research.

The history of the Institute began in 1939 with formation of the Bone Char Research Project at the National Bureau of Standards in Washington, D.C., under the direction of Dr. Victor Dietz. In 1963, it moved to New Orleans, Louisiana, and became the Cane Sugar Refining Research Project, with Dr. Frank Carpenter as its director. In 1981, under Dr. Margaret Clarke, its scope was greatly expanded when it became Sugar Processing Research, Inc. In 1991, it was renamed Sugar Processing Research Institute, Inc. Mary An Godshall became the Managing Director of the Institute in 2000.

SPRI seeks to devote its best efforts to be a center of excellence in sugar technology research and information exchange for the sugar industry through its member companies.

FORWARD

The theme of the 2002 Conference was “**advances in the chemistry and processing of beet and cane sugar.**” A wide range of topics is covered within these pages, reflecting the wide ranging activities and interests of the sugar industry.

The winner of the SPRI Science and Technology Award, Dr. Benjamin L. Legendre, gave a broad overview of the quest for quality in Louisiana sugarcane and sugar, and showed how the interaction between agronomic research and chemistry helped to create stronger insights into changes in sugarcane caused by various variety programs, as well as the impact on raw sugar.

Dr. Luc Moens, of the Center for Renewable Energy, in an invited presentation, discussed sugarcane as a renewable feedstock for the chemical industry and considered the challenges as well as the many opportunities facing the industry in the future, as the world moves toward a carbohydrate economy.

Other topics of discussion include papers on biocides, including the innovative use of hop beta acids and rosin acids, developed for the sugarbeet industry, and applicable to cane as well. Papers on newer technologies include membrane filtration to reduce lime usage in beet sugar purification and the integration of ultrafiltration and ion exchange to produce a new edible product from the mill. Two papers discuss the use of hydrogen peroxide in sugar processing, while three papers discuss cane deterioration, sources of losses, and the search for markers to determine cane deterioration and cold tolerance. A session on colorants, always of interest, in both beet and cane processing, includes five papers. One of the papers, by Dr. Koteeswaran, was included by invitation, although not presented, because it provides new insights on color in cane processing when sulfite is utilized.

The paper entitled, Industrial Economical Optimization of the Juice Extraction Process for Spanish Autumn and Spring Sown Beets, by Marta Garcia de Quevedo and Manuel Ruiz-Holst, received the first Margaret Clarke Best Paper Award.

There were also a series of posters on many topics, ranging from scale composition to vinasse composition.

We hope that the reader will find many interesting ideas in these pages.

Mary An Godshall
December 2002

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2002 SPRI Science and Technology Award

BENJAMIN L. LEGENDRE



Dr. Benjamin Leighton Legendre, Ph.D., a member of the third generation of Legendres involved in sugarcane and sugar production, was born on August 22, 1943, and raised in Thibodaux, Louisiana.

Being raised in the shadow of the sugarhouse and surrounded by vast sugarcane fields on the banks of Bayou Lafourche, Dr. Legendre was inspired to major in agriculture, receiving his undergraduate and graduate degrees from Louisiana State University. He married Nell Louise Talbot and they have four children, Lisa, Ben, Jr., Nicole and Stephanie. He earned his Ph.D. in Agronomy from LSU in 1970, with a specialization in Plant Breeding, and emphasis on sugarcane. Upon graduation, Dr. Legendre served his country in the U.S. Army as a Field Artillery officer, attaining the rank of captain. During his tour of duty in Vietnam, he was

assigned to Civil Operations and Rural Development Support as an Oil Seeds and Feed Grain Advisor. He received the bronze star medal for meritorious service. Upon discharge in 1971, he returned to the USDA-ARS Sugarcane Research Unit at Houma, Louisiana, as a research agronomist. He worked there until 2000, eventually becoming the Research Leader, directing all research activities of the Unit. He retired from Federal government service in 2000 and returned to LSU as the Sugarcane Specialist in the Plant Science Division of LSU's AgCenter. He currently lives on the family's 1,200-acre sugarcane farm in Thibodaux.

Dr. Legendre is recognized as one of the leading sugarcane experts in the world in the areas of breeding and selection, of cane and juice quality, and for his work on plant growth regulators as chemical ripeners. He is also sought out widely for his help to solve milling and processing problems associated with varieties and harvest systems. He has accepted invitations to visit and consult in Argentina, Australia, Brazil, Colombia, Costa Rica, Dominican Republic, Egypt, India, Indonesia, Mauritius, Nigeria, Pakistan, Philippines, South Africa and Thailand. In his current assignment, Dr. Legendre is responsible for all sugarcane educational programs for the State of Louisiana.

SPRI SCIENCE AND TECHNOLOGY AWARD

The SPRI Science and Technology Award is presented biennially to an outstanding scientist, whose research accomplishments are distinguished by their originality and their contribution to sugar processing and production. The Award is presented for the purpose of promoting science and technology in sugar processing and production.

Previous Winners of the SPRI Science Award

- 2000 Jean-François Thibault, Unité de Recherche sur les Polysaccharides, Nantes, France
New Ways to Add Value to Sugar Beet Pulp
- 1998 Markwart Kunz, Südzucker AG, Mannheim/Ochsenfurt, Germany
Sucrose - Raw Material for Chemistry and Biochemistry
- 1996 Pascal A. Christodoulou, Hellenic Sugar Industry, Thessaloniki, Greece
Energy Economy Optimization in the Separation Processes of Sucrose-Water and Non-Sugars
- 1994 Frieder Lichtenthaler, Technical University of Darmstadt, Germany
Computer Simulation of Chemical and Biological Properties of Sucrose, the Cyclodextrins and Amylose
- 1992 Riaz Khan, Poly-Bios, Trieste, Italy
Chemical and Enzymic Transformations of Sucrose
- 1990 Giorgio Mantovani, University of Ferrara, Italy
Growth and Morphology of the Sucrose Crystal
- 1988 Leslie Hough, King's College London, Kensington, London
Sucrose, Sweetness and Sucralose
- 1986 Andrew Van Hook, Holy Cross College, Worcester, Massachusetts (deceased)
Events in Sugar Crystallization

Previous Winners of the SPRI Industrial Technology Award

- 2000 Luis Rocha San Miguel Bento
- 1998 Peter Rein

In 2002, the SPRI Science Award and the SPRI Industrial Technology Award were combined into a single award, the SPRI Science and Technology Award.

President's Welcome and Introductory Remarks

Dennis Costesso
Amalgamated Sugar Company

Thirty years ago, the United States beet sugar industry had eight individual companies with viable operating research programs. The programs were focused on providing each respective company with the competitive edge that comes from reducing costs through technological improvements. These programs not only provided our industry with some very innovative technological advances, but also provided an infrastructure of technologists very knowledgeable in the art of processing sugar.

Today, only one privately held sugar research group is left in all of North America. During the difficult economic times that have transpired over the last thirty years for our industry, doing away with research programs was one of the first cost cutting measures practiced in an effort to control costs. This same trend seems to be taking place in the European beet industry, although to a lesser extent.

Our industry, today seems to be one of technologists improving existing technology. It has been said that there has not been a major change in how sugar is produced since the industry changed from using slaughter house blood to lime for juice purification. Capital dollars today are largely focused, besides on the obvious environmental needs, on making existing unit operations more efficient, mainly by reducing energy and labor costs.

We need to ask ourselves, is simply improving upon existing technology enough to guarantee our future? A quick evaluation of the potential benefits from a typical beet sugar factory using 40% steam on beets, installing state of the art energy and labor reducing equipment, suggests the potential reduction in manufacturing costs is less than one dollar per cwt of sugar. While even this level of cost reduction is helpful during difficult economic times, we must ask ourselves if the level of return from improving existing technology is all we expect for the future of our industry? If not, then how can our capital strapped industry provide the research needed to survive in the future?

As previously mentioned, the approach toward process research in the past has been for each company to have its own dedicated research group. There has been a shift in many manufacturing technologies away from this generally accepted discipline toward one of collaborative research. Collaboration, or working together toward a common goal, has led to some of the most productive and successful technological developments in our history.

Collaborative research is nothing new to our industry. It has been practiced intensely by our agriculture sectors for years. Significant alliances between academia, seed producers and the sugar industry have resulted in numerous successes. Yet it has remained difficult, in many instances, for our industry to make collaboration work in the area of sugar processing research. It must be

acknowledged however, that several of the worlds most cost effective producers of sugar do have successful collaborative research programs in place.

For example, CTC in Brazil has collaborative research programs in place with several local Universities working on projects such as juice treatment, white sugar production, ethanol, water treatment and pollution control. SRI in Australia has as one of its goals to pursue non industry funding for large strategic R&D projects such as bagasse gasification. They are seeking government collaboration to significantly increase the funding of their R&D program.

SMRI in South Africa is also making a special effort to increase collaborative research at the local University level, sponsoring several related post graduate projects.

This same collaborative approach to process research is what your association in SPRI can bring to your industry. As we speak, SPRI has in place a unique collaborative net work that includes not only producers of beet and cane sugar but industry suppliers and sugar users from around the world. SPRI has also increased collaboration with local Universities such as Louisiana State and with the University of Alabama, Center for Green Manufacturing, and has taken the first steps to obtain additional funding through government grant programs. SPRI is in a position of bringing together the best minds of industry, academia and technology manufactures to come up with innovative new processes to help our industry. But they need the continued support of its existing members and the support of new members if they are to continue functioning.

Any industry that is focused on being around for the long term knows how crucial investing in research is. To do otherwise is paramount to giving up on the industry.

SPRI must have the support of industry if they are to continue providing research support and sponsoring conferences for our industry. SPRI was able to put this year's conference on only because of the contribution of its members. While SPRI welcomes and appreciates the attendance of non members at its conferences, it must be stressed again that if SPRI conferences are to continue, it will require everyones participation in SPRI as a sponsoring member.

Please take these thoughts back to the leaders of your companies and help SPRI to help make our sugar industry viable for the long term. I would also ask for the industry to be thinking about research and collaborative ideas and how SPRI can help.

SPRI Science Award Keynote Address

**THE QUEST FOR QUALITY IN LOUISIANA
SUGARCANE AND SUGAR**

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ABSTRACT

Extensive research has shown that sugarcane quality directly affects sugar yield and sugar quality. In many production systems, both agricultural and manufacturing, there is conflict between productivity in the field and factory and quality of sugarcane in the field and sugar in the factory. High productivity and/or throughput compete with high product quality, even with today's emphasis on total quality. However, quality can be influenced by ever-changing developments in sugarcane agriculture including the introduction of new cultivars, use of chemical ripeners, changes in cultural practices and harvesting systems and introduction into an industry of new disease and insect pests. These developments differentially affect the yield of sugar per unit area as well as have a dramatic impact on cane and juice quality and have a direct bearing on the quality of the sugar produced. Research has also shown that these changing developments can have significant deleterious effects on the levels of soluble solids, sucrose content and reducing sugars, concentration of total polysaccharides including starch and dextran, concentration of phenolics and inorganic ash as well as other products of normal metabolism and/or deterioration such as the concentration of manitol, ethanol and various oligosaccharides. Many of these constituents of the juice or cane have a direct bearing on sugar quality as well. There is every incentive to growers in Louisiana, who receive some 60-65 percent of the value of the sugar produced, to deliver good quality material to the processor, who, in turn receives the remaining 35-40 percent. Note that the producer receives a percentage of the value of the sugar, not just a percentage of the actual sugar produced. The value of the sugar or the price paid for raw sugar today goes far beyond just its polarization. Other factors that weigh heavily on the price received for raw sugar include color, ash content and dextran concentration. Experience around the sugar world has shown that operational and moral incentives are not enough to ensure the best available input quality; financial incentives must be provided. In Louisiana processors have gone a step further to ensure better cane quality by providing chemical ripeners to the producer to enhance sucrose and purity of cane at harvest. Further, there are additional financial incentives today that include additional premiums or penalties for cane having above or below average yield of theoretical recoverable sugar or above or below average pol percent cane, fiber

content and sediment (field soil) in the juice. In many instances the incentive given for quality cane has encouraged even higher quality, since producer returns should more than justify the additional expenditures required to achieve this higher quality. This paper summarizes the steps taken by the Louisiana sugar industry in its quest to ensure quality sugarcane and sugar.

PREDICTING CANE QUALITY

Standard Ton Method. The Jones-Costigan Act of May 1934 (Keller and Seip, 1948) was an amendment to the act creating the Agricultural Adjustment Administration (AAA) and put sugarcane and sugarbeet production on a quota basis. It provided for benefit payments to United States producers from revenue derived from a processing tax on imported sugar. However, the benefit payment plan necessitated a purchase contract between processors and growers based on quality of the cane delivered to the factory. Previously, cane was purchased on a weight basis only. Because of this new direction, the Sugar Section of the AAA called on the industry for facts on which to base a sugarcane purchase contract. In September 1934, a meeting of growers and processors produced the first Louisiana Sugar Cane Purchase Contract. This was accepted by the Secretary of Agriculture as the basis for determining the value of sugarcane to calculate the amount of benefit payment necessary to increase grower income to parity. Parity was defined as the equivalence between the grower's current purchasing power and their purchasing power at a selected base period (1909 to 1914) maintained by government support of agricultural commodity prices. A Grower-Processor Committee was organized to supervise contractual relations of growers and processors. Although the benefit payment provisions of the Jones-Costigan Act were nullified by a Supreme Court ruling, the Sugar Act of 1937 reestablished the primary essentials of the former act.

Under the Sugar Act, the basis for cane payment was the standard ton of cane with the expected or theoretical yield of recoverable sugar per ton calculated by applying the Java formula. A standard ton was defined as a net ton of cane with a normal juice sucrose (NJS) and purity (NJP) of 12.0 and 76.5%, respectively. The expected yield of 96 pol sugar at the factory for each standard ton was 160 pounds (72.7 kg) in 1934, increasing to 165 pounds (75.0 kg) in later years. Payment was adjusted upward or downward, depending upon actual normal juice sucrose and purity above or below the standard. Penalties were set at 1% reduction of expected yield of 96 pol sugar for each 0.1% NJS under 12.0% to 11.0% NJS, then 2% of expected sugar yield for each 0.1% NJS under 11% to 9.5% NJS. Premiums were set at one rate: 1% increase of expected yield of 96 pol sugar for each 0.1% NJS over 12.0% NJS. The penalty at 9.5% NJS was 60% of the base value of the cane. No payment was made for sugar if the NJS dropped below the 9.5% mark. Additional penalties and premiums were ascribed when the NJP of the juice was lower or higher than the expected value of NJP for a specific value of NJS.

As long as the cane was hand-cut and hand-stripped, actual yield nearly equaled the theoretical yield based on the Java formula. However, by 1943 there was a considerable discrepancy between the actual and the theoretical yield (for payment). The discrepancy was largely caused by to the drop in normal juice extraction and boiling house efficiency brought about by the change to mechanical harvesting and the increase in trash (extraneous matter) to include cane tops, leaves, weeds and soil delivered with the harvested cane. In 1947, the industry began to measure the amount of trash in

cane deliveries and to make deductions for all trash in excess of 3% of the gross weight of cane. In the first year alone, deductions for trash totaled 127,000 short tons (114,300 metric tons). This figure, while impressive in 1947, represented only about 50% of the total trash actually delivered in the cane to the factories for processing. Trash sampling and testing procedures were far from satisfactory mainly because of the manner of collecting and the size of the sample.

The entire uniform procedure for evaluation of sugarcane to meet the requirements of the Sugar Act was published in the Agricultural Stabilization and Conservation Service Handbook entitled, "Sampling, Testing, and Reporting For Louisiana Sugar Processors" (1968). In general, the procedure stated that sugarcane must be sampled before entering the factory because it was the common practice at that time to wash the cane. One sample of approximately 100 pounds (45.5 kg) was required for every 65 short tons (58.5 metric tons), but not less than one sample per grower per day. The sampling was done with a mechanical grab after the grower's consignment was weighed and placed on the "feeder" table. The entire sample was used to determine trash content, and a second sample (minimum of ten stalks) along with any trash was crushed through a three-roller mill with the juice analyzed for Brix and pol (sucrose). By means of the trash determination, gross cane was converted to net or trash-free cane. The values of the sample mill Brix and sucrose were factored to obtain the normal juice equivalency. Normal juice purity was derived as the ratio of NJS to normal juice Brix (NJB) times 100. Net tons of cane were then converted to standard tons based on the NJS and NJP. The conversion from gross to standard cane in the days of hand-cut and hand-stripped to gross to net to standard tons after field mechanization led to an unwieldy system of questionable accuracy. Further, the method did not take into account the effect of the increased fiber (resulting from the ever-increasing trash) on the factory's extraction and sugar recovery. Legendre and Irvine (1974) reported that each 1% in cane trash increased the fiber content by 0.18 percentage points while reducing extraction by 0.37 percentage points. The net result was a reduction of approximately 3.0 lb (1.36 kg) in the yield of recoverable sugar per ton of cane.

Core/Press Method. As early as the 1950s, Birkett and Seip (1974, 1975) stated that there was a critical need for developing a better method of sampling as a means of providing incentives for growers to deliver better quality cane to the factory. To meet this need, they initiated studies on core sampling and the methods for processing the sample. However, it was not until 1972 that they began a full-scale investigation into an idealized cane sampling system they were to call the core/press method. The goals of an idealized cane sampling system were defined as follows:

1. Remove the judgment factor in selecting and processing the sample.
2. Standardize the sample procurement and processing equipment.
3. Minimize personnel requirements.
4. Divorce the system from the factory operations.
5. Provide a sample representative of the quality of the material in the cane consignment.
6. Reflect the effect of trash in general and field soil in particular on juice quality and quantity.
7. Provide a measure of cane quality in terms of the estimated recoverable sugar in the cane.
8. For the processor, contribute to the factory chemical control and for the grower, provide a means for evaluating varieties, cultural practices, and/or harvesting systems.

All of these prerequisites are afforded by the core/press method of analysis.

The Sugar Act remained in force with only slight modifications until 1974. Even after its expiration, the same purchase contracts remained until the core/press method of analysis was adapted by the Louisiana sugarcane industry. The core/press method was introduced into Louisiana in 1975. Today, all 16 factories in the state are using the core/press method for the determination of cane quality by predicting the yield of theoretical recoverable sugar per ton of cane (TRS) for use in cane payment. The system comprises the following operations:

1. Use of a corer to obtain a cane sample from the delivery of cane.
2. A sample preparation device to prepare the cored sample.
3. A hydraulic press to extract juice from the prepared cored sample.
4. The analysis of the press juice for Brix, pol and percent of sediment and the analysis of the press residue (bagasse) for moisture.

According to Birkett (1977, 1989), the core/press method, as developed for Louisiana, differed from the conventional Louisiana system in three major aspects: sample removal, sample processing and analytical procedures. With regard to sample removal, the cane sample is removed by a corer (a rotating hollow tube with saw teeth on the end) that penetrates the full depth of the cane delivery while drilling through the cane mass in the delivery vehicle. Next, the corer ejects the cane sample into a pre-breaker that prepares the sample for processing. The pre-breaker should achieve a reasonable degree of cane preparation (preparation index of 80% or better is desirable) without removing any juice or drying the sample during preparation. A sub-sample of 2.2 pounds (1 kg) of the prepared cane is pressed in a hydraulic press at 2,500 lb/in² (175.7 kg/cm²) for 1.5 to 3.0 minutes. The hydraulic press separates the cane sample into juice and residue (bagasse), both of which are analyzed, the former for Brix by refractometer and pol (sucrose) by polarimetric measurement and the latter only for moisture (by drying).

In most cases, sugarcane is delivered to the factory with excessive field soil (mud). Tests have shown that 30% of this mud was getting into the extracted juice from the press while 70% of the mud was retained in the bagasse. The effect of these solids in the juice was to inflate the juice content of the cane while decreasing the “fiber” (true fiber plus non-cane solids) content of the cane, resulting in an overestimation of the sugar yield. To reduce the effects on sugar yield, a representative sample of the press juice is centrifuged for 5-10 minutes to determine the sediment volume % juice. To obtain a representative sample, the entire press juice sample as collected from the press should be vigorously stirred to ensure complete suspension of the sediment. Sediment volume % juice is then converted to dry sediment % juice and eventually to weight of extra residue which is added to the residue weight of the sample. The press juice is centrifuged to determine the sediment volume of the juice. This sediment volume is used to correct the residue weight for the quantity of field soil in the juice.

The complete analytical procedures with the derivations for all formulas are described in Legendre (1992) and Birkett (1998). The basic data given to the growers on a computer print-out are their complete cane analyses and their yield of theoretical recoverable sugar per gross ton of cane (TRS). TRS is calculated using the following equation assuming a constant reduced extraction of 91.90% (absolute juice lost % fiber of 56.67) and a Boiling House Efficiency of 96:

$$\text{TRS} = (0.28P - 0.08B) \times [100 - 56.67F / (100 - F)]$$

Where TRS = Theoretical Recoverable Sugar in lb per gross ton of cane at 96 pol
 P = Pol (sucrose) % cane
 B = Brix % cane
 F = Fiber % cane

TRS is then converted to the yield of commercially recoverable sugar per gross ton of cane (CRS) by applying a standardized factor called the Liquidation Factor (LF), which is obtained by dividing the actual factory sugar production for the year by the total TRS calculated for all cane purchased by the factory for the year. Prior to 1998, it was known that there was a tendency for the core/press method to overpredict the TRS in cane by 3-5%; however, this had little or no effect on cane payments since the payments were based on sharing a fixed percentage of the total sugar produced with the growers. It was realized that a true pol (sucrose) % cane would provide better information to growers, factories and researchers; however, the method was more cumbersome and time consuming. Typically, the growers' share of the CRS (TRS x LF) was 60%. Percentage sharing might be a single figure or it may be higher for high quality cane and lower for low quality cane. In recent years, the growers' share has been increased to 61-63% by most factories. The proforma payments for sugar made during the crop, usually each week, are made on the basis of a percentage of the CRS, with the final payment coming after all sugar is sold and delivered to the refinery. Several interim payments are usually made to the growers between the end of the harvest and the time the final adjustment is made. The payment for molasses is independent of the sampling method, with the growers receiving from 50-60% of its value based on the average volume of molasses per ton of gross cane produced by the factory for the crop.

Major changes occurred in 1993 that would revolutionize the Louisiana sugarcane industry and have a far-reaching impact on cane quality and analytical procedures. First, there was the development of a high-yielding sugarcane variety, LCP 85-384, which tended to lodge badly prior to harvest. The variety was quickly expanded by growers, and it was soon obvious that the variety could not be harvested by conventional means, i.e., the whole-stalk harvesting system. Second, the combine harvesting system, which had been in use by many sugarcane industries outside Louisiana, was introduced into the state in 1995. It was soon realized that, to harvest high tonnage and lodged fields of LCP 85-384 efficiently, the industry had to consider converting to the new combine harvesting system. By 2001, approximately 80% of the sugarcane acreage of the state was planted to LCP 85-384 (Legendre 2001), and the same percentage of the crop was harvested by the combine system. However, this switch to LCP 85-384 and the cane combine did not occur without its share of problems.

Prior to the introduction of the combine, the average trash in harvested cane was estimated to be less than 10%; however, with LCP 85-384 and the new combine system, the trash content of harvested cane increased to as much as 20% or more. Further, in lodged fields, a large percentage of cane tops were delivered with the cane to the factories. With this dramatic increase in cane trash, especially leaves and tops, it was determined that the overprediction in the yield of TRS as determined by the core/press method and pol (sucrose) as determined by direct (true) cane analysis could exceed 10%

(Birkett and Stein, 1999). In 1998, Birkett and Stein conducted a series of tests to compare again the core/press method with direct (true) cane analysis and to investigate how the core/press method could be modified to predict cane quality and sugar yield more accurately. As part of the correction in the formula, they suggested converting fiber to fibraque. Fibraque is defined as the Brix-free water that is part of the natural fiber. To calculate fibraque, one simply multiplies the fiber content by a fixed factor of 1.3. This increases fiber content by 30% and also serves to reduce extraction and, consequently, to reduce TRS. Further, there was also an additional correction for pol (sucrose) and Brix of cane (as well as purity % cane), which helped to reduce the overall TRS such that the calculated value for TRS is now similar to the yield calculated by direct (true) cane analysis. Using the fibraque correction, the following calculations are now used to predict the value of TRS:

$$\begin{aligned}\text{Fibraque (New Fiber)} &= \text{NF} = \text{F} \times 1.3 \\ \text{New Pol (Sucrose)} &= \text{NP} = \text{P} \times (100 - \text{NF}) / (100 - \text{F}) \\ \text{New Brix} &= \text{NB} = \text{B} \times (100 - \text{NF}) / (100 - \text{F}) \times \text{Z} \\ \text{Where Z} &= 1.15 - 0.0018 \times (1000 - \text{Corrected Residue Wt.}) / 10\end{aligned}$$

$$\text{TRS} = (0.28\text{NP} - 0.08\text{NB}) \times [100 - 56.67\text{NF} / (100 - \text{NF})]$$

Where TRS = Theoretical Recoverable Sugar in lb per gross ton of cane at 96 pol
 NP = Pol (sucrose) % Cane
 NB = Brix % Cane
 NF = Fiber % Cane

The Liquidation Factor (LF) and conversion of TRS to CRS are obtained in the same manner as described above.

INCENTIVE-BASED QUALITY

Even after the formula adjustments to the Core/Press Method were enacted by all factories for the 1999 crop year, it was still felt by many processors that cane quality was still lacking and two new incentive measures designed to improve the quality of cane delivered to the factory were proposed (Anonymous). However, cane quality was still being defined in terms of recoverable sugar per ton of cane. Two incentive-based methods based on TRS derived from the Core/Press Method using the fibraque correction have been implemented at several factories since the 1999 crop year. They are: 1) TRS – 40 Method and 2) Bonus/Penalty Method (sometimes referred to as the “Sidewalk Formula”).

TRS – 40 Method. In the TRS - 40 Method, the TRS derived from the Core/Press Method for all growers is reduced by 40 lb (18.2 kg). A new LF is calculated for use on a daily basis by dividing the actual yield of commercially recoverable sugar per ton for the day by the average TRS minus the 40 lb for the same day. The new LF will always have a value higher than the LF derived without the 40-lb adjustment. The resulting impact on cane quality and cane payment is that some growers receive less sugar (penalty) and some growers receive more sugar (premium) than actually determined by the Core/Press Method. Cane delivered with a corer TRS below the grower average

for the day would have a payment TRS of less than the corer TRS. In other words, the grower would be penalized for delivering cane with a below-average TRS. On the other hand, cane with a corer TRS above the grower average for the day would have a payment TRS of more than the corer TRS. This grower would actually receive a premium for delivering cane with an above-average TRS. The greater the difference in TRS from the average for the day, the greater will be the penalty or premium. Growers who deliver cane with a TRS at the average for all growers would receive neither a penalty or premium. Examples of the TRS – 40 Method are as follows assuming a mill average TRS for the day of 200.0 lb (90.9 kg) and a Liquidation Factor (LF) of 1.25:

Grower A

$$\text{Corer TRS} = 160.0 \text{ lb (72.7 kg)} - 40.0 \text{ lb (18.2 kg)} = \text{New TRS} = 120.0 \text{ lb (54.5 kg)} \times 1.25$$

$$(\text{LF}) = \text{CRS} = 150.0 \text{ lb (68.2 kg)}$$

Grower B

$$\text{Corer TRS} = 240.0 \text{ lb (109.1 kg)} - 40.0 \text{ lb (18.2 kg)} = \text{New TRS} = 200.0 \text{ lb (90.9 kg)} \times 1.25$$

$$(\text{LF}) = \text{CRS} = 250.0 \text{ lb (113.6 kg)}$$

In these examples, Grower A would be assessed a penalty of 10.0 lb (4.5 kg) while Grower B would be given a premium of 10.0 lb (4.5 kg). Regardless of the penalty or premium, the growers' share of the final CRS figures remains at 61-63%.

The Bonus/Penalty Method. The Bonus/Penalty Method is actually a three-tiered incentive/penalty system that allows for a premium or penalty for each of three criteria - pol (sucrose) % cane, fibraque % cane and sediment - as measured by the Core/Press Method in comparison with the averages of these criteria for all growers. In addition, the adjustment for each criterion is based on a fixed percentage (constant) of the average TRS for all growers. The incentives/penalties in the Bonus/Penalty Method can be adjusted by changing the weighting of the pol (sucrose) % cane, fibraque % cane and sediment and the constant. Initially, it was proposed that the weightings for pol % cane, fibraque % cane and sediment would be 30, 50 and 20%, respectively, and that the constant would be 3% of the average TRS for all growers; however, both the weightings and/or constant have been changed by several factories. An example of how this method works is as follows assuming a mill average TRS for the day of 200.0 lb (90.9 kg) and a constant multiplier of 3% or 6.0 lb (2.7 kg) ($200.0 \text{ lb} \times 0.03 = 6.0 \text{ lb}$) and a Liquidation Factor (LF) of 1.01:

For Grower A

$$\text{Pol \% cane} = 2.5 \text{ units lower than average pol (sucrose) \% cane of all growers} \times 0.30$$

$$(\text{Weighting}) \times 6.0 \text{ lb} = -4.5 \text{ lb (2.0 kg) } \mathbf{\text{penalty}}$$

$$\text{Fibraque \% cane} = 1.5 \text{ units lower than average fibraque \% cane of all growers} \times 0.50$$

$$(\text{Weighting}) \times 6.0 \text{ lb} = +4.5 \text{ lb (2.0 kg) } \mathbf{\text{bonus}}$$

$$\text{Sediment} = 0.5 \text{ units lower than average sediment of all growers} \times 0.20 (\text{Weighting}) \times 6.0 \text{ lb} =$$

$$+0.6 \text{ lb (0.3 kg) } \mathbf{\text{bonus}}$$

$$\text{Total } \mathbf{\text{bonus/penalty}} = -4.5 \text{ lb (for pol \% cane)} + 4.5 \text{ lb (for fibraque \% cane)} + 0.6 \text{ lb (for sediment)} = +0.6 \text{ lb (0.3 kg)}$$

$$\text{Corer TRS} = 160.0 \text{ lb (72.7 kg)} + 0.6 \text{ lb (0.3 kg)} (\text{Total bonus}) = \text{New TRS} = 160.6 \text{ lb (73.0 kg)}$$

kg) x 1.01 (LF) = CRS = 162.2 lb (73.7 kg).

Again, regardless of the penalty or premium, the growers' share of the final CRS figures remains at 61-63%. For this example, even though the grower's pol (sucrose) % cane was 1.5 units lower than the all-grower average, the grower still received a slight premium for having cane with lower fibraque % cane and sediment below the average of all growers. For the Bonus/Penalty Method, information pertinent to the grower's sample could appear on the grower's printout from the factory for each criterion to provide justification for the bonus and/or penalty received. Recommendations are also made to the grower for possible improvement. A stated advantage of this method is that a grower might have some control over one or more of the criteria that affect TRS.

The real justification for the development of the Core/Press Method was its accurate valuation of cane that encouraged quality with increased profitability to both growers and processors. However, not even the use of the Core/Press Method with its modifications has there been sufficient impetus to achieve the quality of cane needed by the Louisiana industry to remain competitive. Further, the incentive formulas enacted to date have done little to improve cane quality and, in many instances, have caused a strain in grower-to-grower and grower-to-processor relationships.

DEVELOPMENTS IN SUGARCANE AGRICULTURE THAT AFFECT QUALITY

According to Clarke and Legendre (1996), there can be a conflict between productivity and quality. New innovations and/or the need for high throughput compete with high product quality, even with today's emphasis on total quality. While cane and juice quality have, undoubtedly, suffered from the increase in extraneous material delivered with the cane for processing, the refineries and end users are demanding higher quality raw and refined sugars, respectively. Although growers receive payment based on tons of cane across the scale and the yield of commercially recoverable sugar per ton of cane, the sale of sugar to the refinery is based on more than just weight and polarization. Today, purchase contracts for both raw and refined sugar consider the following: color, ash, moisture, safety factor, turbidity/sediment, reducing sugars, grain size, filterability and dextran. Refiners are also concerned with starch, gums and other polysaccharides that have a negative impact on the refining process.

Godshall, *et al.* (2000) stated that there is a two-fold objective in cane harvesting and processing – to maximize sucrose and to minimize impurities. To maximize sucrose, high yielding, disease resistant varieties are used, field and processing conditions are optimized and microbiological contamination is controlled. To minimize impurities, there is the need for clean, fresh cane.

Impact of varieties on selected quality parameters. Research has shown that significant differences in the chemical composition of sugarcane varieties (Legendre, *et al.*, 1999) include starch, polysaccharides, proanthocyanidins and phenolics that have been shown to affect processing and sugar quality. Starch in sugarcane is normally found at highest concentration in immature cane, in the leaf blade and in the nodes. Starch becomes soluble in processing when the juice temperature exceeds 71°C. Rather than being eliminated in the filter cake during clarification, it remains in the

clarified juice and concentrates in the syrup as the juice is evaporated where it increases viscosity and inhibits crystallization along the way. The amylase form, in particular, slows the boiling rate, which leads to higher energy usage, increases the molasses volume with a corresponding loss of sugar, and is found in raw sugar which can cause serious problems at the refinery. A study conducted by Godshall *et al.*, (1996) also showed that sugarcane varieties differ significantly in the concentration of starch with a range of 275 to 1,460 ppm (on solids) for the sugarcane varieties CP 70-321 and CP 72-370, respectively. Table 1 shows the starch concentration of 3 of the 10 varieties included in the study. Godshall *et al.* (2000) stated that the transfer of starch in juice to starch in sugar ranges from 30-50%.

Table 1. Impact of varieties on starch concentration of juice ^{1, 2}

Variety	Starch Concentration (ppm on solids)
CP 70-321	275 a
CP 76-331	647 b
CP 72-370	1,460 c

¹ From Godshall *et al.*, 1996.

² Means in a column followed by the same letter are not significantly different at the 95% confidence level.

Polysaccharides, which can include starch and dextran as well as other large carbohydrate molecules, have several important functions in the sugarcane plant. Polysaccharides are hard to remove in process and delay crystallization (Legendre *et al.*, 1999). They are found in the raw sugar and may contribute to color formation, thus adversely affecting sugar quality and increasing the cost of processing and refining. Godshall *et al.* (1996) showed that sugarcane varieties also differ significantly in the concentration of polysaccharides with a range of 1,455 to 3,234 ppm (on solids) for the sugarcane varieties CP 70-321 and CP 72-370, respectively. Table 2 shows the total polysaccharide concentration of 3 of the 10 varieties included in the study. From 20-30% of the total polysaccharide found in cane juice are found in the raw sugar (Godshall *et al.*, 2000).

Table 2. Impact of varieties on total polysaccharide of juice ^{1, 2}

Variety	Total Polysaccharide (ppm on solids)
CP 70-321	1,455 a
LCP 82-89	2,161 b
CP 72-370	3,234 c

¹ From Godshall *et al.*, 1996.

² Means in a column followed by the same letter are not significantly different at the 95% confidence level.

Proanthocyanidins are related to plant pigments and appear to be associated with sugarcane polysaccharides (Legendre, *et al.*, 1999). Proanthocyanidins have yet to be associated with processing difficulties. Godshall *et al.* (1996) found a significant difference in the concentration of proanthocyanidins among 10 varieties studied which ranged from 10 to 65 A₄₈₅ x 1000 for CP 74-383 and CP 72-370, respectively. Table 3 shows the proanthocyanidins concentration of 3 of the 10 varieties in the study.

Table 3. Impact of varieties on proanthocyanidin pigments of juice ^{1, 2}

Variety	Proanthocyanidin Pigments (A ₄₈₅ x 1000)
CP 74-383	10 a
CP 82-551	31 b
CP 72-370	65 c

¹ From Godshall *et al.*, 1996.² Means in a column followed by the same letter are not significantly different at the 95% confidence level.

Phenolics are important from a processing standpoint because of their reactivity with metal ions and ability to produce highly colored reaction products (Legendre, *et al.*, 1999). As much as two-thirds of the color in cane juice may be caused by enzymatic browning of phenolic acids. Because of their ability to change color with pH value, the presence of phenolic compounds in raw sugar can strongly affect color measurement. Godshall and Legendre (1988) reported that there was a significant difference in the concentration of phenolics among four varieties studied. Table 4 shows the phenolic concentration of 3 of the 4 varieties studied. The phenolic concentration ranged from 393 to 686 µg/ml (mean caffeic acid equivalents) for the sugarcane varieties CP 70-321 and CP 65-357, respectively. In this study, the concentration of phenolics increased with the maturity of the crop (data not shown). Further, phenolic constituents accounted for as much as 0.4% of the refractometric dry substance of the juice in trash-free cane. However, as the level of trash increases, the concentration of phenolics would also increase, further exacerbating the problem. Godshall *et al.* (2000) reported that from 10-20% of the color in cane juice is found in the raw sugar.

Table 4. Impact of varieties on phenolic concentration of juice ^{1, 2}

Variety	Phenolic Concentration (mean caffeic acid equivalency in µg/ml)
CP 70-321	393 a
CP 74-383	542 b
CP 65-357	686 c

¹ From Godshall and Legendre, 1988.² Means in a column followed by the same letter are not significantly different at the 95% confidence level.

Use of Chemical Ripeners. Although plant breeding and selection of sugarcane varieties have been responsible for the rapid increase in yield of recoverable sugar per unit area, sugarcane growers still depend largely on natural environmental conditions to trigger the maturation process (Legendre *et al.*, 1999). As an alternative to reliance on climatic factors affecting natural ripening, regardless of variety, and with cognizance of the need to harvest immature sugarcane in Louisiana, growers now use the chemical ripener glyphosate to enhance the yield of recoverable sugar per ton of cane by 5-30%, depending upon the variety (Legendre, 2001). Glyphosate has also been shown to reduce the fiber content of cane by 3-5%, which has a positive impact on extraction and recovery of sugar. Glyphosate formulations have been shown to improve the sucrose content as well as purity, which translate into an increase in the yield of commercially recoverable sugar per ton of cane over a wide

range of climatic conditions, are less variety-specific and the ripening response induced in the sugarcane is more consistent and rapid than that obtained with most other ripeners (Table 5).

Table 5. Effect of glyphosate on increasing yield of commercially recoverable sugar per ton of cane of selected varieties recommended for planting in Louisiana ^{1, 2}

Variety	Increase in Commercially Recoverable Sugar per Ton of Cane (lb)
CP 70-321	9
LCP 85-384	36
HoCP 85-845	12
HoCP 91-555	22

¹ From Legendre, 2001.

² Glyphosate was applied at 0.3 kg/ha in 190 l of spray mixture and cane harvested at 49 days after treatment.

The use of glyphosate does not come without potential adverse effects on processing, namely the decrease in reducing sugars-to-ash ratio and a possible increase in both the concentrations of polysaccharides and dextran, which can ultimately contribute to processing difficulties and lower sugar quality. However, in two studies conducted in 1985 and 1986, the concentration of dextran (ppm on juice) was numerically higher in treated cane harvested 56 days after treatment but not significantly different from the control fields not treated with glyphosate (Legendre *et al.*, 1999). Further, it was noted that no differences were detected in dextran concentration when converted to ppm on solids. Because of the direct benefit to the processor in improving both sucrose content and purity of cane, all factories in Louisiana contribute to the cost of the application either in payment for the product or the cost of the aerial application or both.

Topping Height. It has been recognized since 1935 that the topping height has considerable effect upon the quality of juice from harvested sugarcane (Arceneaux, 1935). Mechanical harvesting with the whole-stalk or "soldier harvester" led to the concept of "average topping" rather than the older practice of topping each stalk at the uppermost mature internode when cane was cut by hand (Coleman, 1959). However, in high tonnage and severely lodged cane little, if any, topping is practiced when cane is harvested by combine harvesters. When sugarcane stalks were topped too low by the whole-stalk harvester, mature cane on the taller stalks was left in the field, resulting in a loss of cane yield. However, when the stalks were topped too high, immature cane on the shorter stalks would be delivered to the factory with a resulting loss of sugar yield (Davidson, 1965). Godshall *et al.* (2000) compared the results for several components of sugarcane juice originating from clean stalks and leaves and tops. In every case, leaves and tops caused a reduction in juice quality when compared to the juice derived from the clean stalks.

Table 6. Impact of leaves and tops on juice components ¹

Component	Juice from Clean Stalks	Juice from Leaves and Tops
Brix (%)	18 – 22	7 – 10
pH	5.44	5.34
Color	6,280	77,660
Indicator Value (I.V.)	5.54	2.73
Polysaccharide (ppm)	1,352	20,044
Starch (ppm)	710	4,037
Filtration rate (10ml/0.45μ)	15 minutes	130 minutes

¹ From Godshall *et al.*, 2000.

Legendre (1996) showed that both sucrose and purity of cane increased with the severity of topping while reducing sugars were highest in cane with intact tops. The concentration of reducing sugars decreased with the severity of topping. Significant differences were also noted in inorganic ash with the severity of topping with higher ash in juice with no topping (Legendre *et al.*, 1994) (Table 7). It was also shown in these studies that there were significant differences in the inorganic ash % total solids among varieties. In south Texas, concentration of ash in extracted juice is used as a selection tool in variety development (Roseff, personal communications).

Table 7. Effect of topping height on the concentration of inorganic ash % total solids in extracted juice of three varieties ^{1, 2}

Variety	Topping Height (Level of Topping) (Inorganic ash % total solids)			
	None	10 cm Above the Bud	40 cm Above the Bud	Mean
CP 65-357	0.56 b	0.45 ab	0.41 a	0.48 B
CP 70-370	0.63 b	0.52 ab	0.46 a	0.52 B
CP 72-370	0.48 b	0.37 ab	0.34 a	0.40 A
Mean	0.56 b	0.45 a	0.40 a	0.47

¹ From Legendre *et al.*, 1994.

² Means followed by the same letter in rows (lower case), read across, and in columns (upper case), read down, are not significantly different at the 95% confidence level.

High ash content is associated with lower factory efficiency and sugar recovery and contributes to a higher volume and purity of final molasses with a significant loss of sugar. Godshall *et al.* (2000) stated that approximately 5-10% of the ash in juice will remain in the sugar.

Changes in harvest systems. According to de Beer (1998), reduced labor availability and very small profit margins mandated mechanization of the sugarcane handling process. Further, de Beer and Purchase (1998) stated that it is unfortunate that as field operations are mechanized, field losses increase and the quality of the product delivered to the factory for processing decreases. For both manual and mechanical harvesting of sugarcane, the condition of the harvested crop has a significant effect on the quality of cane delivered to the factory (Legendre, 1981). Typical quality factors

include the maturity of the crop, quantity of tops and leafy trash, number of suckers, dead cane, roots and soil.

de Beer and Purchase (1998) listed the advantages of green cane harvesting on raw sugar factory operations. They are as follows: 1) greater overall recovery of sugar per unit area of land, 2) stale cane reduced to a minimum with the onset of rainy weather that may delay or stop the harvest entirely, and 3) greatly reduced dextran levels in juice and, therefore, sugar. However, green cane harvesting also has its disadvantages. They are: 1) more extraneous matter (trash) in harvested cane, 2) greater concentration of waxes, ash and starch in extracted juice, increased molasses production, increased sugar losses per ton of cane and reduced milling capacity resulting in increased cost per unit of sugar produced, and 3) a higher concentration of color in juice. Of course, many of these constituents will carry over into the refinery, reducing refined sugar quality. It is imperative that agronomic practices be closely aligned to harvesting requirements since adverse agronomic practices cannot usually be completely offset by improved harvester design or harvesting practices. Although the combine harvester can harvest cane green, many growers are burning standing cane, where permitted, prior to harvest to reduce the amount of trash in cane while increasing the efficiency of the harvester.

According to Eggleston *et al.* (2001), the recent increase of billeted cane being harvested in Louisiana has often meant an increase in deterioration in cane quality that is, the increase in associated trash is not necessarily a function of the newer harvest method, *per se*, but rather a function of mechanical harvesting in general. Further, there is the occurrence of sugar destruction in the cut cane between harvesting and crushing, regardless of the harvest system. There is a real need to establish new criteria to measure deterioration in Louisiana harvested cane to better predict: 1) the quality of the cane to be processed and 2) the effect of harvest methods and storage conditions.

Trash. As industries increase their level of mechanization, lower cane quality is often observed with an increase in trash; however, overall efficiency is normally improved, and costs are often reduced (Richard, 1999). Paton (1997) summarized results of research conducted in Australia, which found that the switch to mechanized and green cane harvesting as opposed to hand harvesting of burned cane was responsible for the increased trash (extraneous matter) in cane delivered to the factory resulting in lower sugar yields. He also noted that new, high-yielding varieties have a greater tendency to lodge and produce an abundance of “water shoots” or suckers that contribute to the increased extraneous matter while contributing little sugar. Another factor to consider is the use of increased fertilizer inputs with the new varieties that further contribute to lodging and reduced sugar yield (Kennedy *et al.*, unpublished data). Approximately 80% of the sugarcane crop in Louisiana is now harvested by combine to take advantage of the new variety, LCP 85-384, which is prone to lodging. However, the amount of trash in harvested cane cut by combine is significantly higher when compared with cane harvested by hand (Legendre and Richard, 1997). The combine harvester chops the cane stalks into billets of 17 to 35 cm long and, with proper operation of extractor fans, can remove a significant portion of the leafy trash without burning (de Beer *et al.*, 1996) (Table 8). In 1997, Legendre and Richard as reported by

Table 8. Trash content of green and burned sugarcane harvested by the cane combine under different extractor fan speeds in South Africa ¹

Crop	Fan Speed	Trash
	(RPM)	(%)
Green	1,000	10.6
	1,250	8.5
	1,450	7.4
Burned	1,000	6.6
	1,250	5.2
	1,450	3.7

¹ From de Beer *et al.*, 1996.

Legendre *et al.* (1999) found similar results when operating the cane combine at different fan speeds under Louisiana conditions. However, little or no topping is practiced in lodged cane resulting in increased trash in the cane consignments. Harvesting cane under wet field conditions generally results in a higher level of both field soil and trash in cane consignments. As stated earlier, it has been shown that for every 1 percent in trash (either leafy trash or field soil) there is a reduction in the yield of commercially recoverable sugar of approximately 1.4 kg (3 lb) (Legendre, 1975 & 1991; Legendre and Irvine, 1974).

Deterioration. Research has shown that burned and/or chopped cane deteriorates faster than whole-stalk cane regardless of whether it was cut by hand or machine (Table 9). Further, it has been shown that chopped cane should be processed within 12-20 hours of harvest because of the formation of dextran produced by the bacterium *Leuconostoc mesenteroides* (Irvine and Legendre, 1974 & 1977; Legendre *et al.*, 1999). The presence of dextran indicates specific loss of sucrose. Clarke (1996) stated that the major factors affecting dextran levels in raw sugar are related to field and harvest conditions. These factors include: 1) ambient temperature, 2) ambient humidity, 3) rainfall before and at the time of harvest and the amount of mud, 4) degree of burn of cane, 5) integrity of the cane stalk or billet (extent and severity of damage to the stalk), 6) billet or stalk length, 7) delay between burning and cutting, and 8) delay between cutting and crushing. With increasing time, dextran concentration increases asymptotically while with increasing temperature and humidity, dextran concentration of the juice increases dramatically. Sugarcane varieties may also have an effect on the susceptibility to *Leuconostoc* infection while in the temperate climates where sugarcane is grown, freeze damage to the stalk tissue can also have a strong influence on the concentration of dextran.

The best control for dextran is to inhibit its formation. A major aspect of control is the coordination of harvest schedules with factory deliveries and crushing. Good coordination of harvest and delivery with minimum holding times after burn or harvest are important for all cane, but especially for combine-harvested or billeted cane (Legendre, 1990). Dextran can be formed at the factory, first in the cane yard, and second, in juices and waters containing sucrose all across the mill tandem until the juices are heated. Control of *Leuconostoc* and dextran production in the factory is best accomplished by good hygiene, good housekeeping and the judicious use of bacteriostatics. However, if dextran is already present in the juice because of poor quality cane or mill hygiene, control may be accomplished by either treatment by dextranase enzyme or removal in syrup clarification.

Table 9. Effect of burning and harvest system on the deterioration of sugarcane (variety, CP 70-321) measured by the concentration of dextran in extracted juice ^{1,2}

Treatment	Dextran Concentration (ppm on Brix)	
	Whole-stalk (Soldier Harvester)	Billeted Cane (Combine Harvester)
Unburned Stalks		
Control	64 A	95 A
1 DAH	109 A	1,850 B
2 DAH	312 A	3,423 D
Burned Stalks		
Control	153 A	253 A
1 DAH	166 A	2,968 C
2 DAH	276 A	3,155 CD

¹ From Legendre and Richard, 1997 as reported in Legendre *et al.*, 1999.

² Means followed by the same letter in columns are not significantly different at the 95% confidence interval.

Dextran causes numerous problems in processing including increased viscosity, crystallization time and energy use, elongation of the sucrose crystal along the C-axis, and, most important, loss of sugar with increased molasses volume in both the raw cane sugar factory and the refinery (Aikins and McCowage, 1984; Clarke and Legendre, 1996). Godshall *et al.* (2000) reported that approximately 30-50% of the dextran in juice will be transferred into the raw sugar crystal.

Eggleston *et al.* (2001) reported that deterioration in storage occurs much earlier and faster in billeted cane than in hand-cut or soldier-harvested whole-stalk cane. They also noted that burned, billeted cane deteriorates faster than green, billeted cane in storage, as indicated by a greater decrease in pH, and a greater increase in titratable acidity, color, invert and dextran concentration with time in storage. They found that billeted cane can deteriorate rapidly after the first 24h of storage (although deterioration begins before 24h), which reinforces the need for factories to process billeted cane as quickly as possible. They noted that, because of the rapid increase in dextran, the source of the deterioration is, most likely, microbial.

Color. With the changes in harvest systems, the amount of extraneous material has increased. In South Africa (de Beer and Purchase, 1998), it was reported that with each 1% addition of tops to clean cane the color of clear juice as measured by absorbance was increased by 1.3% while with each 1% addition of mud to clean cane the color of clear juice was increased by 3.6%. Subsequent studies by Purchase *et al.* (1991) showed that trash contributed substantial color and turbidity to juice. They found that trash appeared to contribute more color than tops alone but when results were normalized there was a linear relationship between color in juice and extraneous material.

In Australia, Ivin and Doyle (1989) defined trash as incorporation of the remains of leaves attached to the cane stalk but not including field soil. Further, tops were defined as that portion of the cane stalk above the break point approximately 25 cm in length, minus the top leaves normally cut and blown clear by the chopper harvester. They found that juice color increased an average 25% with the

addition of 6% trash (i.e., a 4.2% increase in color for every 1% of trash). They also noted a 12% increase in juice color by the addition of 6% green tops (a 2% increase for every 1% tops). However, the increases in juice color were highly dependent on the variety of cane as well, with tops contributing from as little as 2% color in one variety and as high as 29% color in another. The range for trash effects alone on color by variety was from a low of 9.8% to as high as 47.6%.

Legendre *et al.* (1996) investigated the effect of leafy trash and soil on color. They found more than a 6-fold increase in color caused by leafy trash over the treatment range studied (0 to 30% trash) (Table 10). There was a 13% increase in juice color for every 1% leafy trash added, up to the 10% level, which was within the range of the Australian experience (Ivin and Doyle, 1989). Field soil alone showed a decolorizing effect, undoubtedly because of the ion exchange properties of the soil type (Sharkey clay) (Legendre *et al.*, 1996). In a follow-up study, Godshall *et al.* (2002) stated that more than one soil series has the ability to remove color (as well as polysaccharide) from cane juice, to improve filterability, with ash level remaining unchanged, or slightly decreased, and no deleterious effect on pH. In the first study, the effect of soil was linear with an approximately 1.6% decrease in color per 1% of added soil up to the 30% level. The results for the combination effects of leafy cane trash and mud mixture reflect the opposing effects of the two components. These results further show that the components of trash can have different effects on cane juice color, and it is important to define the composition of trash.

Table 10. Average value for ICUMSA color of centrifuged cane juice determined at 420 nm in the presence of various admixtures of leafy trash and mud (variety CP 70-321) ¹

Admixtures of Leafy Trash and Mud (% of Gross Weight of Cane)	ICUMSA Color (420 nm)		
	With Mud Added Only	With Leafy Trash Added Only	Mixture of Mud and Leafy Trash Added
0 (Clean Cane)	2,910	2,910	2,910
10 or 5 & 5	2,554	6,665	6,208
20 or 10 & 10	1,799	12,148	7,084
30 or 15 & 15	1,315	19,432	7,645

¹ From Legendre *et al.*, 1996.

Godshall *et al.* (2002) stated that their work on soil was not intended to advocate or recommend bringing soil in with harvested cane. Soil has destructive effects on the mills, increases the burden to the clarifier and contributes to disposal costs. They stated that the results are of considerable interest because they can help explain some anomalous behavior in cane juice quality when there is a lot of mud brought to the factory. It may be possible, in the future, to consider how to exploit the beneficial effects of the soil in the cane-growing area of Louisiana. However, in the meantime they still feel that the cleaner the juice, the better in the long run.

Eggleston *et al.* (2001) reported that color and invert concentrations of sugarcane juice and, undoubtedly, the sugar are mostly attributable to the amount of “green” leaves and cane tops being delivered in cane consignments. These results are similar to those reported by Godshall *et al.* (2000) in an earlier study where they found that the greatest differences occurred between green and burned cane, regardless of method of harvesting.

Disease and insect complexes. Plant phenolic concentration (color) has been associated with fungal, bacterial and insect resistance (Cruikshank and Perrin, 1964; Friend, 1979). Many fungal diseases of sugarcane, notably red rot, but also *Fusarium* wilts and rots, *Helminthosporium* eye spot and others, induce the sugarcane plant to produce phenolic pigments (Edgerton, 1959). It is possible that an increase in phenolic concentration may also be a general stress response caused by mechanical and chemical injury, sugarcane borer damage and bacterial and viral disease-induced tissue reddening.

Sugarcane Yellow Leaf Virus. Grisham *et al.* (2001) presented evidence that sugarcane yellow leaf virus (SCYLV) could reduce cane and sugar yield even when symptoms of yellow leaf syndrome (YLS) were not present. They also found that YLS increased soluble solids (brix) and starch in the juice extracted from leaf tissue having the disease (Table 11). They stated that this is a concern in sugar processing since it may result in a loss of recoverable sucrose and an increase in molasses. With mechanical harvesting, the leaves and tops of many stalks are often not properly separated from the stalks and enter the milling process. Further, with the introduction of the combine harvester and the increase in the harvesting of unburned cane in Louisiana, more leafy trash and tops are being delivered to the factories. The results of this study suggest that green leaves and tops of sugarcane yellow leaf virus-infected plants may have a direct impact on cane and juice quality, and may affect boiling house efficiency.

Table 11. Impact of sugarcane yellow leaf virus (SCYLV) on the starch concentration of juice from green sugarcane leaves (variety LCP 82-89)¹

Crop Year	Starch Concentration (ppm)	
	SCYLV Positive	SCYLV Negative
Plant-Cane Crop	4,477	2,772**
First-Ratoon Crop	1,951	1,489**
Second-Ratoon Crop	4,777	1,840**

¹ From Grisham *et al.*, 2001.

** Differences between infected (positive) and control (negative) plants significant at P = 0.01.

SUMMARY AND CONCLUSIONS

This paper summarizes the steps taken by the Louisiana sugar industry in its quest to ensure quality sugarcane and sugar. It describes cane payment methods and developments in sugarcane agriculture that affect quality. Experiences around the world have shown that operational and moral incentives are not enough to ensure the best available raw material for the factory, and financial incentives must be provided. As early as 1934, sugarcane growers in Louisiana were paid on the basis of cane

quality where previously cane was purchased on a weight basis only. The “Standard Ton Method”, as it was called, was based on the quality of the normal juice coupled with trash deductions. However, as early as the 1950s, it was realized by the industry that there was a critical need for developing a better method of sampling as a means of providing incentives for growers to deliver better quality sugarcane to the factory. In 1975, a new procedure was introduced called the “Core/Press Method”. It was based on direct cane analyses for Brix, pol (sucrose) and fiber in cane, which provided a measure of cane quality in terms of the estimated recoverable sugar in the cane. Adjustments to the core/press method were introduced at the beginning of the 1999 harvesting season, which reduced the differences between the yield of theoretical and commercially recoverable sugar per ton of cane. However, even after the adjustments, it was felt by many processors that cane quality was still lacking, and two incentive measures designed to improve cane quality were proposed. The incentive-based modifications were implemented by several mills beginning in 1999 that provided for a premium and/or penalty for above average and below average quality, respectively. Both incentive-based modifications were based on the yield of theoretical recoverable sugar per ton of cane as predicted by the core/press method.

Although growers now receive payment based on tons of cane across the scale and the yield of commercially recoverable sugar per ton of cane, the sale of sugar to the refinery is based on more than just weight and polarization. Several changes in sugarcane agriculture have been shown to affect juice and sugar quality. These include the introduction of new varieties, the use of chemical ripeners, changes in cultural practices and harvest systems and the introduction of new disease and insect pests into an industry. However, much research is still required to fully qualify and quantify the impact that these agricultural operations have on processing. In conclusion, production and harvest management are keys to optimizing cane quality, and production, harvest and post-harvest management are keys to juice and sugar quality.

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Invited Presentation

SUGAR CANE AS A RENEWABLE FEEDSTOCK FOR THE CHEMICAL INDUSTRY: CHALLENGES AND OPPORTUNITIES

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ABSTRACT

The extraction of sucrose from sugar cane generates large volumes of bagasse as a low-value by-product for which very limited uses exist in today's market. The challenges associated with the fractionation of bagasse into its cellulosic and lignin components have made it very difficult to establish uses for bagasse as a feedstock material for chemicals production. However, the last two decades have witnessed the development of a new method for fractionating lignocellulosics, as well as the establishment of chemical technologies that allow for the conversion of cellulose into distinct 'platform chemicals' in higher yields than before. This convergence of better technologies creates opportunities for the development of new and economically viable routes for converting bagasse into higher-value platform chemicals. This paper will discuss three significant platform chemicals that can be generated from cellulosic fractions in biomass, i.e. levoglucosan, levulinic acid and 3,4-dihydroxybutyric acid. Future work should make it possible to derive these platform chemicals from sugar cane bagasse.

INTRODUCTION

In spite of the widespread use of sucrose as a commodity for the food industry, the sugar producing industry in the United States remains under tremendous economic pressure as a result of global competition. Intense efforts towards the chemical conversion of sucrose into new chemicals and materials have met with only limited and often short-lived success. The inherent attractiveness of sucrose as a chemical stems from its unique, highly functionalized molecular structure. The polyol character of this disaccharide has been the source of many synthetic pathways towards useful compounds, and its chiral properties continue to stir the imagination of many chemists around the world.¹ However, the real large-scale use of sucrose in synthetic processing schemes has yet to be realized in an economically attractive manner. In addition,

much of the proposed and tested chemistry with sucrose involves the glucose or fructose of which it is composed, and these simple sugars can be derived in pure state from a host of other agricultural commodities such as e. g. corn. Consequently, the search for higher-value products within the sugar industry should focus on the by-products that are generated in large volume during the sucrose extraction process, and for which today only a limited number of market outlets exist. Especially in light of the progress that has been made over the last two decades in the area of biomass chemistry and processing, a good target would be bagasse, which is the lignocellulosic residue that remains after all sucrose has been extracted from sugar cane.^{2,3} The large volume of this material that is generated annually (ca. 8 million STRV)⁴, combined with its significant cellulose content (30-40%), make bagasse a viable biomass feedstock for the production of high(er)-value chemicals and materials according to new and improved processing techniques.

The concept of breaking a complex feedstock down into smaller chemical building blocks has been the focus of the petroleum industry since its inception. After nearly two centuries, this industry has reached a level of maturity that allows it to produce a spectrum of so-called '*platform chemicals*' (petrochemicals) that serve as chemical feedstocks for the chemical industry as we know it today. In essence, the chemical industry has developed the necessary 'tools' to increase the degree of oxygenation of the platform molecules (hydrocarbons) such that a plethora of commodity and fine chemicals can be generated along economically viable pathways. Unlike fossil feedstocks, lignocellulosic biomass is much more complex and variable in chemical composition, and has a significantly more pronounced chemical reactivity due to its high degree of oxygenation. In contrast with the well-established thermal steps for 'cracking' petroleum fractions in an oil refinery, a '*biorefinery*' would consist of a number of novel and 'alternative' chemical technologies for processing biomass.⁵

The purpose of this paper is to highlight a few key developments that have taken place over the last two decades in the arena of chemical conversion of cellulosic biomass into platform chemicals. Some of the technologies that will be discussed here have been part of the chemical R&D activities in our laboratories. This stems from our interest in the development of new chemical technologies for converting renewable biomass as part of a search for alternative energy and carbon sources. Since cellulose is the most abundant natural polymer, we have a strong focus on the use of this material as a source for either chemicals or for the production of larger molecular weight components for advanced materials. Consequently, we envision new possibilities for generating platform chemicals from the cellulosic fraction in bagasse, and thus higher-value market outlets for this by-product may be created.

Even though microbial or biochemical conversion methods are gaining importance in the processing of carbohydrates, this paper will focus on platform chemicals that can be produced under thermal conditions or through acid- or base-catalysis, and with high product selectivities, minimal generation of by-products and low energy consumption.

Clean Fractionation of Biomass

One of the primary challenges with any type of lignocellulosic biomass is its fractionation (pulping) into three main components, i. e. cellulose, hemicellulose and lignin. Better known as

'pulping', such a separation process is practiced for the production of cellulose in the papermaking industry, but a huge industry also exists for the conversion of cellulose into chemicals, plastics, food additives, fibers and textiles. Over 1.5 billion pounds of chemical grade cellulose are used annually for the production of almost 2.5 billion lb/yr of cellulose derivatives. One of the largest uses of chemical grade cellulose (>300 million lb/yr) is in the production of cellulose esters, materials that are used in such diverse applications as photographic films, cigarette filters, tool handles, and clothing. In contrast to cellulose used in other applications, such as paper manufacture, the cellulose produced for chemical applications normally has much higher performance requirements. For example, cellulose used in the preparation of cellulose esters must exhibit a purity of greater than 97%. Conventional pulping technology exhibits low cellulose yields (35-40%) and malodorous emissions. An important limitation is that the conventional separation is *only* useful for the production of cellulose. The lignin and hemicellulose fractions are generally not obtained in an easily useable form. This means that two potentially valuable co-products, if they are isolated, are relegated to very low value applications such as fuel or animal feed, which constitutes a poor overall effectiveness in the use of the biomass resource.

To address these shortcomings, NREL has developed a new method for the separation and purification of chemical grade cellulose called *Clean Fractionation (CF)* (Fig. 1). This work has resulted in significant improvements in the way that chemical grade cellulose can be isolated and purified.⁶ The cellulose exhibits performance properties in the production of cellulose esters that meet or exceed those of industrial standards. Importantly, the process also makes clean lignin and hemicellulose streams for chemical production. The NREL CF process is a new example of a broader technology known as 'organosolv fractionation'. In this process, the cellulosic feedstock is treated with a ternary mixture of methyl isobutyl ketone (MIBK), ethanol and water in the presence of a dilute acid promoter such as sulfuric acid, and the mixture is heated at 140°C under one hour. The method can be applied to a wide spectrum of lignocellulosic biomass materials, but the solvent ratio must be optimized for each type of biomass species, and is chosen such that the mixture remains as a single liquid phase at all times during the separation process. The solvent mixture selectively dissolves the lignin and hemicellulose components, leaving the cellulose as an undissolved material that can be washed, fiberized, and further purified. The soluble fraction containing the lignin and hemicellulose is treated with water to cause a phase separation into an organic phase containing the lignin and an aqueous phase containing the hemicellulose-derived sugars.

Thus, greater than 95% of the biomass components present in the starting feedstock can be isolated after fractionation. The CF process normally gives a cellulose yield of about 47-48 wt% in comparison to maximum yields of about 40% in conventional pulping processes. The NREL CF process also allows for 99% recovery of the organic solvents and there are no odorous emissions. Overall, the process produces three renewable raw materials, each of which can be used for the production of chemicals: the cellulose and lignin are isolated as solids, and the hemicellulose fraction is obtained as an aqueous solution of pentoses.

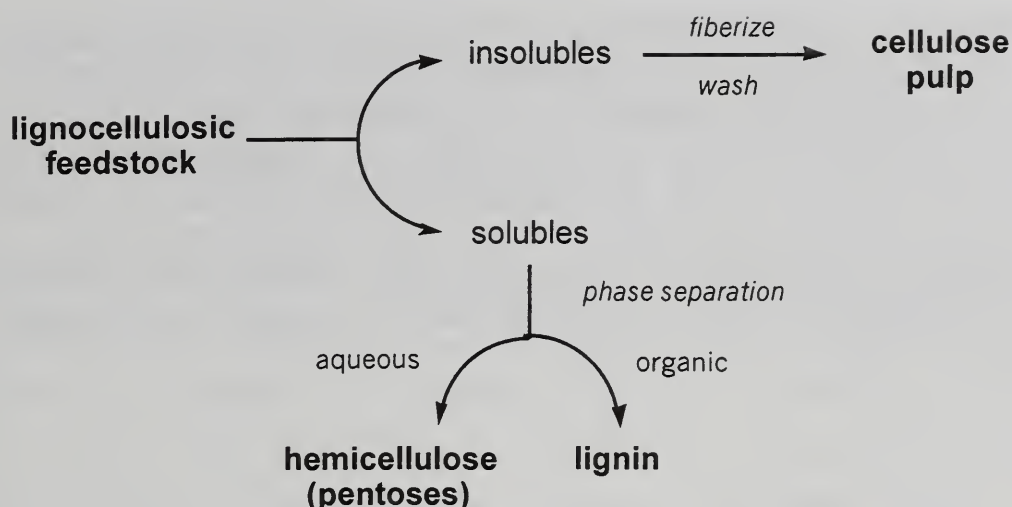


Figure 1. Clean Fractionation Process developed at NREL

Clearly, the CF process represents a new ‘tool’ for producing pure types of cellulose from biomass, and its versatility suggests that it should be applicable to bagasse in an equally successful manner. The challenge will be to optimize the CF process for this particular lignocellulosic feedstock. A preliminary test of the CF conditions on cane bagasse has shown that clean cellulose can be obtained beside a fraction of pentoses (hemicellulose). Although we have not yet determined the properties of the cellulose fibers, we have indication that the cellulose can be generated in high purity such that it could serve as a chemical feedstock for higher-value cellulose derivatives. This, combined with the isolation of hemicellulose-derived pentoses in a separate aqueous fraction, provides new opportunities for the use of cane bagasse as a chemical feedstock.

We also noticed that, generally, the CF process generates lignins that are soluble in regular solvents. Our hypothesis is that the lignins are broken down to smaller molecular weight units that under the CF conditions do not undergo secondary condensation reactions. This is in contrast with e.g. kraft pulp wherein the resulting lignins are subjected to much harsher conditions that lead to their consumption in secondary condensation reactions. The resulting lignins have been found to be much higher in molecular weights and have extremely poor solubility in almost any solvent system. Thus, CF lignins are much better candidates for further chemical processing, and their potential application in the production of fine chemicals should be examined.

Bagasse as a Source of Platform Chemicals

Bagasse is composed of 30-40% cellulose, 20-30% pentosans (hemicellulose), and about 20% lignin.^{2,3} Therefore, the use of novel pulping conditions should make it worthwhile to fractionate sugar cane bagasse into clean and higher-value cellulose and hemicellulose fractions. Aside from the unique material properties of cellulose fibers, the usefulness of this biopolymer may be expanded significantly upon conversion into platform chemicals that have a smaller molecular weight, but that retain some of the rich chemical functionality that is so characteristic of

carbohydrates. A drawback is that the high degree of oxygenation not only increases the chemical reactivity of the intermediates, but conventional chemical catalyst systems may not operate efficiently with these complex substrates. This explains why biomass chemistry and processing has progressed relatively slowly and why, today, only a few significant platform chemicals can be produced efficiently.⁵ In the following sections, we present a series of platform chemicals that have been produced in very good yields from lignocellulosic feedstocks under a variety of process conditions. While these platform molecules have distinctly different chemical properties, they will underscore the rich chemistry that one can develop from cellulosic and hemicellulosic fractions.

Levogluconan

In contrast with the hydrolysis of cellulose in dilute aqueous acid solutions that leads to the formation of glucose, pyrolysis of cellulose leads to the formation of a glucose molecule wherein the C1- and C6- position are connected by an ether linkage (Fig. 2). The resulting hexose is called levoglucosan, or 1,6-anhydro- β -D-glucopyranose, and is a sugar that can be isolated as a highly pure crystalline material.⁷

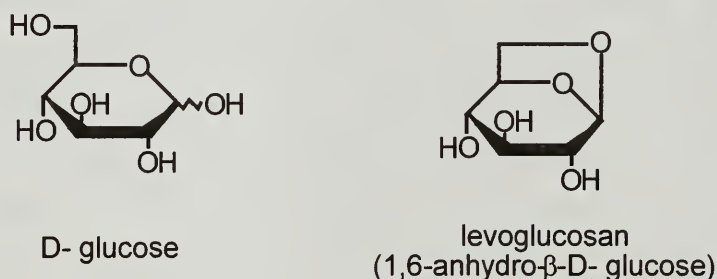


Figure 2. *Structural comparison between D-glucose and levoglucosan*

The yields have been reported to be among the highest (20-30 wt%) of any single compound that has ever been isolated from biomass pyrolysis oils. The highest yields have been observed using Fast Pyrolysis technology (450-600°C; residence time ca. 1s) and were part of studies aiming at the production of biofuels from lignocellulosics.⁸ This technique has made it possible to achieve liquid yields of up to 70-80%, but the recovery of levoglucosan is challenging and has inspired considerable R&D activity worldwide during the 1990s.^{5,7,8} In spite of the many elegant methods for isolating and purifying levoglucosan, these steps are still not completely reliable and economical. Levoglucosan is available commercially as a fine chemical from chemical vendors, but its cost price is so high (\$30/g or higher) that it precludes its use as a chemical starting material for large-scale processes. Nevertheless, levoglucosan remains an attractive molecule for synthetic chemistry because the unique anhydro group makes the ring structure much more rigid than that of a normal glucose molecule, which allows for more selective chemistry with the remaining free hydroxy groups.⁷

There is ample evidence that the yields of levoglucosan are increased after an acid pretreatment of the cellulosic feedstock, while alkali metal ions catalyze the formation of hydroxyacetaldehyde at the expense of levoglucosan formation.⁵ None of these observations are

completely understood, and this complicates the reliability of the process for commercial application. Ideally, some of the by-products of the thermal decomposition process (e.g. acetol, formic acid, hydroxyacetaldehyde, formaldehyde and acetic acid) could be sold as a credit towards lowering the cost of levoglucosan, but the recovery of such by-products is still very challenging and will require more R&D work.

The pyrolysis of sugar cane bagasse has been documented, but primarily for the production of bio-oils.⁸ Although the production of levoglucosan from cane bagasse represents an interesting opportunity, the commercial success will depend on the development of economical and more reliable recovery methods for this unique carbohydrate. In addition, it is crucial that more large-scale market outlets be created for levoglucosan. For instance, levoglucosan has been demonstrated on laboratory scale to be useful for the synthesis of pharmaceuticals, pesticides, oligosaccharides, carbohydrate-based surfactants, and liquid crystals.⁷ Also, a variety of polymerization reactions that lead to unique stereoregular polymers, biocompatible polymers and epoxies have been developed. Some of this polymer chemistry exploits the interesting feature of levoglucosan that it can undergo uncatalyzed polymerization reactions based on ring opening of the ether linkage. Considering that the synthesis of levoglucosan from simple glucose is a very complicated process, the thermal decomposition of cellulose represents the best route towards the production of this platform chemical. The thermal conversion of bagasse-derived cellulose may therefore lead to a number of specialty chemicals and materials, and would fit within the concept of a biorefinery.

Levulinic acid

Levulinic acid, or 4-oxopentanoic acid, is a five-carbon (C_5) compound that is formed through acid-catalyzed (e.g. sulfuric acid) hydrolysis of cellulose.^{9,10} There is evidence that the hydrolysis process involves the formation of 5-hydroxymethylfurfural (5-HMF) that immediately undergoes hydrolysis to form equimolar amounts of levulinic acid and formic acid (Fig. 3). Levulinic acid (LA) is a stable ketoacid that can be isolated in yields and purities that vary strongly depending on the production process. In pure state, it is a crystalline product with a melting point around 30°C.

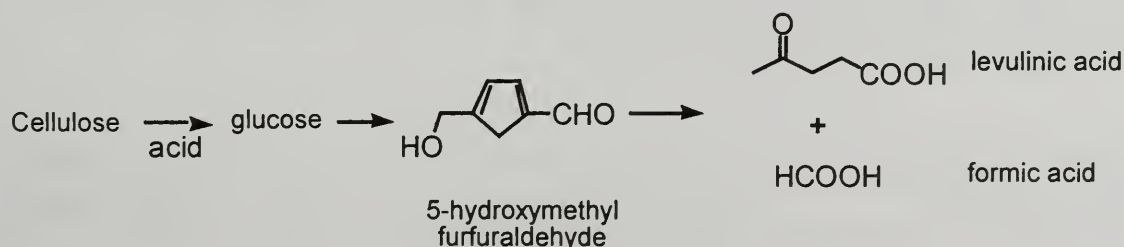


Figure 3. Hydrolysis of cellulose to levulinic acid

LA was at one time made from biomass, but is now produced from maleic anhydride and other petrochemicals. At a price of about \$5.00 per pound, LA has a small, 1×10^3 lb/yr market as a relatively minor commercial chemical that is currently used chiefly as a catalyst in the manufacture of specialty adhesives, rubber, pharmaceutical, plastic, and synthetic fiber products.

By developing a means to produce it very inexpensively from abundant and renewable biomass materials, however, a whole range of new chemical industries derived from LA may become economically attractive. Until a few years ago, large-scale production of this reactive chemical from lignocellulosic biomass was challenging because only low yields (in the range of 20-40% of the theoretical conversion of cellulose) could be obtained due to secondary condensation reactions that resulted in the formation of resin-like materials. However, during the 1990s, new industrial R&D work by Biofine Inc. led to an improved process that uses high-temperature, dilute-acid hydrolysis in a special reactor wherein the newly formed levulinic acid is rapidly removed from the acidic reaction medium to avoid the detrimental secondary reactions. Pilot-scale experiments have shown that yields of 70-90% can be achieved starting from a variety of cellulosic feedstocks, including low-grade cellulose fiber and wastepaper. Biofine has now built a plant that produces 600 pounds of LA per day from 1 ton per day of paper-mill sludge. At this scale, the production cost is about \$2.00 per pound of LA, comparable to current commercial operations (Table 1). However, as the production scale increases, costs decrease dramatically. For example, with a plant size of 50 dry tons per day of feedstock, the cost drops to about \$0.32 per pound, making Biomass LA suitable as a platform chemical building block for a wide range of chemical products in addition to LA itself. Biofine is currently planning large-scale plants that would process about 500 tons per day of paper-mill sludge. The LA from these plants (about 110 million pounds per year) will cost only about \$0.10 per pound (cash cost to manufacture). At this cost, LA could be a platform chemical for a wide range of chemicals derived from cellulosic wastes and "home-grown" sustainable biomass instead of finite, fossil fuels. It should be emphasized that the 'quality' of the cellulose material (i.e. crystallinity, molecular weight etc.) is not important for LA production, since low-grade cellulose fiber in paper-mill sludge can be used as a feed material on large scales.

The development of an improved process immediately led to a strong program to find economical pathways for converting this platform chemical into a variety of useful chemicals (Fig. 4). The products that were recognized as good short-term targets for R&D included a group named *LA Chemicals™*: a) methyltetrahydrofuran (MTHF), an inexpensive industrial solvent, new gasoline extender and oxygenate, and a major component of an alternative fuel; b) delta-aminolevulinic acid (DALA), an economical, highly effective, and environmentally benign herbicide that also exhibits photodynamic antitumor activity; c) diphenolic acid (DPA), a new building block for plastics and coating materials.

Table 1.

Competition Matrix Levulinic Acid (LA)	LA Chemicals™ LA (Biofine Process)	Maleic Anhydride LA (DSM, Neth.)
Cost	\$2/lb @ 1 ton per day \$0.32/lb @ 50 tons per day \$0.10/lb @ 500 tons per day	\$3/lb @ 3 tons per day
Price	\$3/lb*\$1/lb*\$0.25/lb	\$5/lb
Feedstock	Paper-mill sludge or other biomass waste—need not be dried	maleic anhydride, other petrochemicals

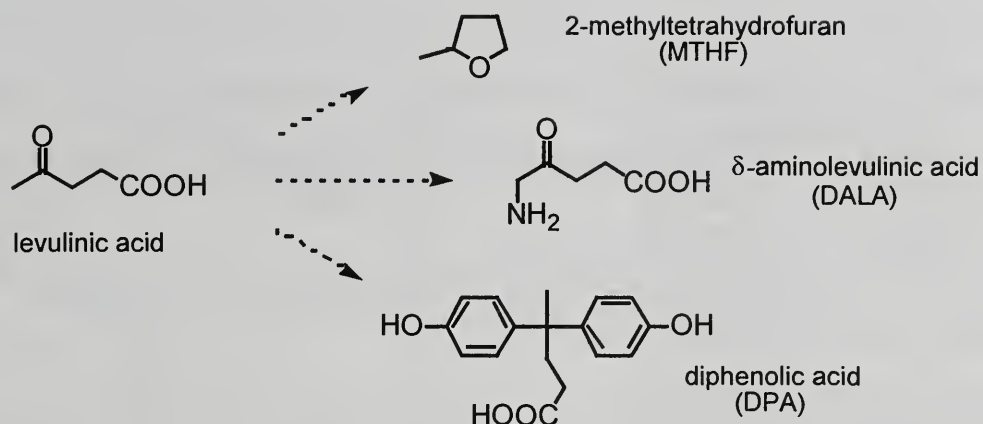


Figure 4. LA Chemicals™

To illustrate the versatile spectrum of products that can be derived from a platform chemical like LA, we will focus on these three LA Chemicals™, highlighting how they may find an immediate entry in the current chemical and fuel market.

Methyltetrahydrofuran (MTHF) is currently produced from furfural produced from corn cobs and sells for up to \$20 per gallon. New R&D work conducted at Pacific Northwest National Laboratory (PNNL) under the program spearheaded by Biofine, has resulted in the development of a patented process that produces about 63 pounds of MTHF per 100 pounds of LA. It has been estimated that conversion of 500 tons per day of paper-mill sludge will yield 12 million gallons per year of MTHF. The PNNL process produces MTHF from levulinic acid by a catalyzed reaction with hydrogen gas. The reaction pathway includes several lactone and diol intermediates and involves the reaction of three molecules of hydrogen for each molecule of levulinic acid. This single-step, single-vessel, catalytic hydrogenation process is highly selective for MTHF and will produce MTHF at a cost of less than \$1.00 per gallon.

Petrochemically derived tetrahydrofuran [THF] enjoys a 500 million lb/yr market, about 70% as a monomer for making polytetramethylene ether glycol [PTMEG] elastic fiber (marketed as Spandex® or Lycra®) and 30% as an industrial solvent. At a price of \$1.00 to \$1.50 per pound (\$7.00 to \$10.00 per gallon), THF is currently in very high demand, with the PTMEG market increasing rapidly. MTHF is not suitable for making PTMEG, but will substitute very well for THF for most industrial solvent uses. As a solvent, MTHF has environmental advantages over THF that can be quite significant. Because MTHF has lower water solubility than THF, it is easier to recover from waste streams than is THF. Because it has a higher boiling point than THF (85°C vs. 65°C) it also generates fewer VOC emissions. Using the PNNL process to make MTHF from LA that is produced on a scale of 500 tons per day of feedstock, solvent-grade MTHF can be made for about \$0.20 per pound (\$1.50 per gallon), which is less than 20% of the

cost of making THF. Even at a production scale as low as 50 tons per day, MTHF could be made from Biofine LA for about \$0.50 per pound (\$3.70 per gallon) and be quite competitive with THF as an industrial solvent (Table 2).

Table 2.

Competition Matrix Methyltetrahydrofuran (MTHF) as solvent	LA Chemicals™ MTHF (PNNL Process)	MTHF from furfural	Tetrahydrofuran
Cost (solvent grade)	\$1/gal (500-tons-per-day-feedstock LA)	\$12-15/gal	\$5/gal
Price (solvent grade)	\$1.50/gal	\$20/gal	\$7-10/gal
Feedstock	paper-mill sludge or other biomass waste-derived LA	corn cobs	petrochemicals
Suitability for making plastics	does not polymerize		excellent for PTMEG (Spandex®)
Suitability for use as an industrial solvent	excellent		good

MTHF is also highly suitable as an automotive fuel extender or oxygenate. Its 18% oxygen level is comparable to that of methyl *tert*-butyl ether (MTBE), and its energy density and octane level are comparable to those of gasoline. MTHF has low vapor pressure, so blending with gasoline is effective in reducing volatile organic compound (VOC) emissions. Fleet tests in Florida in the early 1990s for mixtures with gasoline from 10% to 50% MTHF showed it to perform very well both for vehicle operation and for air emissions. Using MTHF together with ethanol as gasoline additives works particularly well. The MTHF acts as a co-solvent for the otherwise volatile ethanol, resulting in VOC emissions substantially lower than with just ethanol. At a large-scale production cost of less than \$1.00 per gallon, fuel-grade MTHF is competitive with both ethanol and MTBE, the two common fuel oxygenates. The low solubility of MTHF in water gives it a very significant advantage over MTBE, which has been widely blamed for pollution of water supplies, because of its high affinity for water. (Table 3)

In addition, MTHF is a key ingredient in one of the most innovative alternative fuels developed. Pure Energy Corporation of New York has received U.S. Department of Energy approval of their P-Series Fuel formulation as an alternative fuel in accordance with the Energy Policy Act of 1992 (EPAct). EPAct mandates that federal, state, and alternative-fuel-provider fleets use alternative-fuel vehicles. P-Series Fuels can be used in any alcohol-compatible engine, including most flexible-fuel vehicles already available, so they should be very attractive to many fleet operators. The formulation for P-Series Fuels is approximately equal portions of pentanes from natural gas liquids, ethanol, and MTHF. If Pure Energy Corporation successfully markets its P-

series fuels—already approved as an alternative fuel for fleet use—this could use huge amounts of MTHF.

Table 3.

Competition Matrix Methyltetrahydrofuran (MTHF) as fuel additive	LA Chemicals™ MTHF (PNNL Process)	MTHF from furfural	Methyl Tertiary Butyl Ether	Ethanol
Cost (fuel grade)	\$0.50/gal (1000-tons-per-day-feedstock LA)	\$12-15/gal	\$1/gal @ 150 million gallons per year	\$0.50–\$0.80/gal
Price (fuel grade)	\$0.85/gallon	\$20/ gallon	\$1.20/gallon	\$1.20/gallon \$0.66 after subsidy
Feedstock	paper-mill sludge or other biomass-waste-derived LA	corn cobs	petrochemicals	corn starch
Oxygen content	18%		18%	30%
Octane	same as base gasoline		booster	booster
Reid Vapor Pressure (impact on VOC emissions)	reduces VOCs in gasoline formulations, especially mixed with ethanol, reducing ethanol's effect on RVP		increases slightly (8 psi)	increases unless use more than 22% ethanol (18 psi)
Miscibility with gasoline	yes	yes	yes	no
Energy density	same as gasoline		18% less than gasoline	35% less than gasoline

Delta-Amino Levulinic Acid (DALA) is a naturally occurring substance with the potential for very large uses, most notably as an environmentally benign herbicide for use on lawns and for certain grain crops.⁵ Up until now, only uneconomical means have been available to make DALA, so its production has been in small amounts at costs that precluded its use as an herbicide. The current price, estimated at about \$50 per gram when produced from amino acids, limits DALA to uses such as medical treatment that can tolerate the high price. Because DALA can play a similar role in animal cells to what it does in plant cells, medical researchers are currently testing photodynamic cancer therapy that uses DALA. Previous attempts to produce DALA directly from LA, produced only low yields and large amounts of waste products. Also under a cooperative research agreement for Biofine, the National Renewable Energy Laboratory (NREL) has now developed a three-step process to synthesize DALA directly from LA.¹¹ The well-known bromination of levulinate ester to 5-bromolevulinate ester is followed by a nucleophilic substitution reaction using sodium diformylamide as a new aminating agent. This

crucial amination reaction is more atom efficient and amenable to scale-up than previously known methods. Free DALA is then produced by a hydrolysis step with formation of a minimum amount of waste. By increasing yield and purity while using simpler reagents, the NREL process brings the cost of DALA down to \$2 per pound, based on Biofine LA that is produced at 50 tons of feedstock per day. This makes the cost of applying DALA about \$6.00 to \$8.00 per acre. Most lawn and crop herbicides now cost anywhere from \$10.00 to \$25.00 per acre to apply (more for individual lawns), so DALA has a significant cost advantage in addition to being natural and nontoxic (Table 4).

Table 4.

Competition Matrix Delta-Amino Levulinic Acid (DALA)	LA Chemicals™ DALA (NREL Process)	Amino Acid DALA	2-4-D, and other Lawn Weed Killers	Roundup®, Atrazine, and other Crop Herbicides
Cost	\$2/lb (50 tons-per-day LA feedstock)			
Price	\$3/lb	\$50/g		varies
Application Rate	2 pounds per acre			varies
Application Cost	\$6–8 per acre	prohibitive	\$20–25 per acre	\$10-15per acre
Feedstock	paper-mill sludge or other biomass-waste-derived LA	conventional LA	petrochemicals	petrochemicals
Applicability	all dicotyledon weeds (generally any broad leaf plant—most except grasses) alternative formulation kills all plants		dicotyledon weeds	most all except crop designed for
Suitability for turf farms, golf courses, lawns	excellent		excellent	poor
Suitability for grain crops	excellent		poor	excellent
Biodegradability	yes, within day or two		no, long life cycle	varies; Roundup® is more degradable than most
Toxicity to animals	none, abundant in green vegetables		yes	yes
Weed kill rate at optimum dosage	90%-100%	100%	high	high

The mode of action of DALA as an herbicide is rather unique because it is also a naturally occurring substance present in all plant and animal cells. As the precursor molecule for tetrapyrroles such as chlorophyll and the heme of hemoglobin, DALA plays a key role in such vital processes as photosynthesis and oxygen transport. At night, plants gradually convert DALA into chlorophyll and other tetrapyrroles. If DALA is externally applied, however, the metabolic balance is disturbed and an excess of tetrapyrrole intermediates builds up within the plant cells. In the morning, as soon as the plants are exposed to daylight, the accumulated tetrapyrroles use the sunlight to convert oxygen into singlet oxygen that kills plant cells by excessively oxidizing the cell material. Because DALA is completely metabolized, no unnatural residues remain. DALA that is not taken up by the plants decomposes within a day or two, so toxicity and impact on the environment are minimal. In general, DALA is only effective on dicotyledons or broadleaf plants. Monocotyledons such as grasses and grains are able to tolerate it. As such, DALA is ideal for killing broadleaf weeds in grass such as in turf farms, golf courses, and residential lawns. Similarly, it can be used for monocotyledon crops such as corn, wheat, oats, and barley. DALA requires use of small amounts of chemical "modulators" to trigger its herbicidal action. By varying the modulator, DALA can be formulated to fit a variety of situations of weeds to be killed and grass or crop to be left unharmed. The University of Illinois has patented the use of DALA as an herbicide, including a variety of formulations suitable for different crop situations, and Biofine has licensed that use.

Diphenolic acid [DPA], which is easily made by treating LA with phenol under acid-catalysis, is a direct beneficiary of the breakthrough in LA production.^{12,13} Like LA, it is a commercial chemical that will be far more attractive at the lower cost allowed by LA ChemicalsTM technology. DPA was passed over for a major commodity chemical role because it could not compete in production cost with a newer, cheaper petrochemical product. It is still used in some protective and decorative finishes, but in recent years its use has been largely replaced by bisphenol-A (BPA), a less expensive chemical made from acetone and phenol. BPA has also developed a very large market as a key raw material for epoxy resins and other polymers such as polycarbonate, engineering plastics, and polyester. DPA is a suitable monomer for these polymers, but at its previous price of about \$3.00 per pound, it could not compete with BPA, which sells for about \$0.90 per pound. At large scale, DPA can be made from LA from Biofine's process for about only \$0.40 per pound assuming a production volume of 100 tons per day using Biofine LA at 500 tons of feedstock per day (Table 5). It is estimated that this will allow DPA to readily capture about 20% of the huge 1.5 billion lb/yr BPA market.

The New York State Energy Research and Development Authority contracted Rensselaer Polytechnic Institute to investigate the use of DPA and DPA/BPA combinations for polyester, polycarbonate, and other polymers. This work indicates that DPA or DPA/BPA blends can quite effectively substitute for BPA. The blends will have different properties from straight BPA (the DPA molecule has an additional functional acid group), but these differences will for the most part be advantageous, as plastics producers are looking for distinguishing features for new products.

Table 5.

Competition Matrix Diphenolic Acid (DPA)	LA Chemicals™ DPA	Conventional DPA (not commercially available in U.S.)	Bisphenol-A
Cost	\$0.30/lb (LA @ 500 tons per day feedstock)	\$2/lb	\$0.50/lb @ 200 tons per day
Price	\$0.65/lb	\$3/lb	\$0.80/lb
Feedstock	paper-mill sludge or other biomass-waste-derived LA and phenol	conventional LA and phenol	acetone and phenol
Suitability for polyester, polycarbonate, and other polymers	good alone, or in combination with BPA		good
Suitability for epoxy	good in combination with BPA		good
Suitability for coatings	good		good
Suitability for specialty resins	good		poor

These cost analyses clearly demonstrate that LA Chemicals™ MTHF, DALA, and DPA are all far less expensive than these chemicals have previously been, making it possible for them to be used on a large scale for the first time. In addition, if Biofine LA is used as starting material, the entire suite of chemicals is derived from renewable feedstocks. Any number of other chemicals could, however, prove to be economically viable when made from low-cost LA. A few other examples of chemicals that can be made from LA include levulinate esters, metal levulinate salts, n-methylpyrrolidone, 1,4-butanediol, succinic acid, pyrrolidine, ketals, acrylic acid, acetic acid, formic acid, and furans. Many more can be found by perusing the review articles on levulinic acid chemistry that are known in the chemical literature.

This discussion on levulinic acid illustrates the versatile nature of this particular cellulose-derived platform chemical. The purpose was, of course, to highlight a platform chemical that one should be able to produce efficiently and in high yield from a waste material such as sugar cane bagasse. With the advent of very cost-competitive LA, combined with the high chemical functionality of this material, the possible market for its derivatives is expected to expand, and thus also the demand could increase for a variety of agricultural waste materials that could include bagasse.

3,4-Dihydroxybutyric acid

During the last decade, it was found that O-1,4-linked aldohexoses could be cleaved oxidatively into (S)-3,4-dihydroxybutyric acid [(S)-3,4-DBA] in high yields using mixtures of aqueous base (e.g. NaOH) and hydrogen peroxide.¹⁴ This reaction generates glycolic acid as a by-product, and thus a C₆-sugar is cleaved into a C₄-and C₂-unit (Fig. 5). Since the oxidation process leads to formation of the metal salt of S-DBA, the free acid form can be obtained by acidification. However, this free acid form has a strong propensity towards cyclization and can easily be converted into the lactone form.

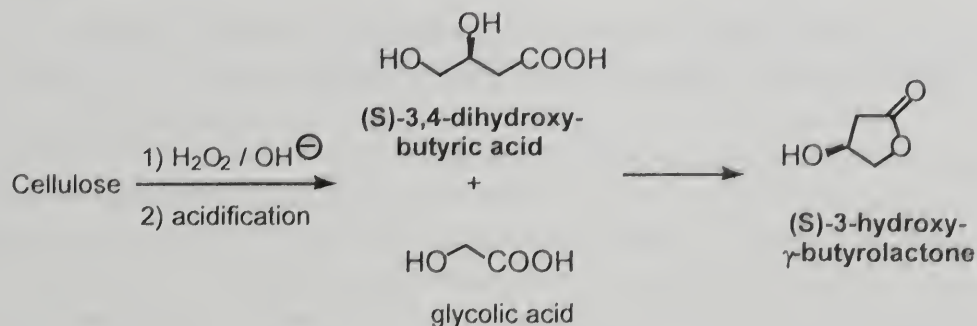


Figure 5. Formation of (S)-3,4-dihydroxybutyric acid from cellulose

The process can be applied to a variety of hexoses, including e.g. glucose and galactose, but also biopolymers like starch or cellulose. Unfortunately, most of the DBA chemistry is confined to the patent literature and the exact yield for the cellulose conversion is not clearly indicated. Nevertheless, it should be interesting to apply this oxidative cleavage reaction to cellulose derived from sugar cane bagasse. An interesting feature is that a relatively inexpensive renewable feedstock can be converted into the optically active and highly functionalized (S)-3,4-DBA, and this opens up new routes to a wide variety of useful chiral building blocks for fine chemicals production.¹⁵

Both free (S)-3,4-DBA and its lactone can be considered to be useful monomeric building blocks for new polymeric materials such as polyesters. Even though the lactone easily undergoes dehydration, the cyclic structure should be useful for ring opening polymerization reactions if mild conditions could be developed such as those that are based on enzyme catalysis (Fig. 6). It would be interesting to investigate how the optically active dihydroxyacid structure may create polymers that are a variant of e.g. polylactide, polyhydroxybutyric acid and polyhydroxyvaleric acid.¹⁶

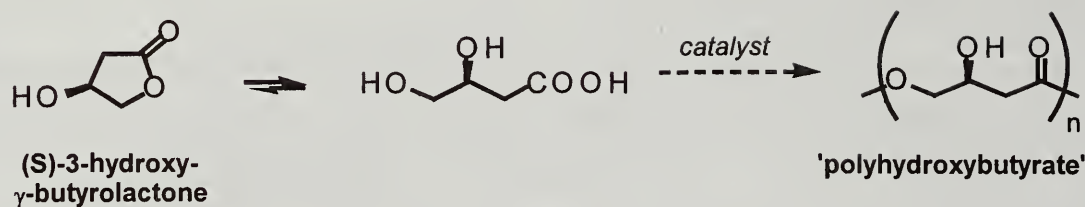


Figure 6. Polymerization of (S)-3,4-DBA may create a new type of polyesters

Another promising aspect of this process is that partially protected L-sugars such as acetals of xylose and arabinose can be converted into (R)-3,4-dihydroxyacid or its corresponding (R)-lactone (Fig. 7).¹⁷ Not only does this provide an entry into the chiral chemistry of the (R)-series, but also any hemicellulosic pentose stream could become a feedstock for this process. Since the Clean Fractionation process allows the production of pentose-rich streams from lignocellulosic biomass, it should be possible to derive some new chemicals from sugar cane bagasse through the isolation and oxidative treatment of its hemicellulose fraction. Obviously, if the pentoses need to be protected in the form of their acetals, it will be crucial to find process conditions that will make this step unnecessary.

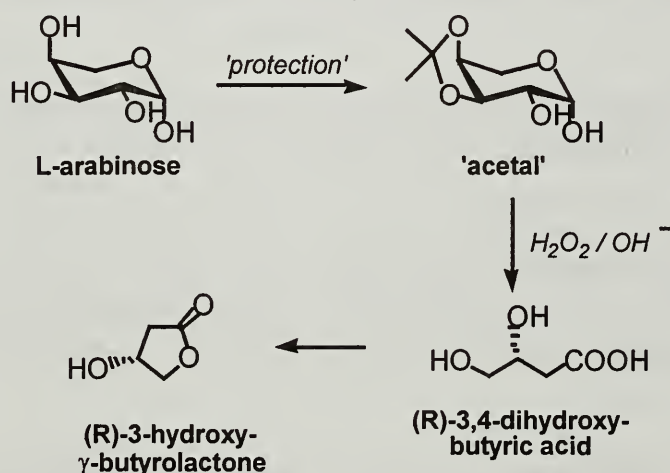


Figure 7. Conversion of L-arabinose into (R)-3,4-DBA and its lactone.

CONCLUSION

Sugar cane bagasse is a lignocellulosic by-product of sucrose production, and increasing its value as a chemical feedstock will hinge upon the success of separating the cellulose, hemicellulose and lignin fractions. The Clean Fractionation process may become a powerful tool to accomplish this goal, even though this process will have to be fine-tuned for bagasse as a feedstock. Once the individual fractions are obtained, a variety of chemical technologies can be utilized to convert these fractions into well-defined platform chemicals. Cellulose can be thermally converted to levoglucosan, or can be hydrolyzed to levulinic acid using process conditions that

have been thoroughly investigated. Especially levulinic acid should receive much more attention since it is such a versatile and reactive molecule from which numerous derivatives can be synthesized. Regarding the production of 3,4-dihydroxybutyric acid from biomass, this chemical appears to be the center of ongoing R&D work. Only very limited information is available in the open chemical literature at this time, probably because of intellectual property issues. Nevertheless, this building block holds much promise not only because of its chemical functionalities, but also because it provides a potentially new market outlet for the hemicellulose fractions for which there is very little commercial use today.

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SOLID PHASE MICROEXTRACTION (SPME) FOR EVALUATING THE SENSORY QUALITY OF SUGAR

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ABSTRACT

Refined cane and beet sugars may still contain trace levels of some undesirable off-odors, which diminish the value of the commodity and limit its use. This research describes methodology for detecting the presence of short chain fatty acids, which have been linked to the off-flavor compounds in sugar. Although not the sole cause of the problem, their presence at trace levels indicates possible problems and their presence at higher concentrations are problematic. Headspace analysis was performed employing solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS). The analytical parameters of incubation temperature, moisture content, and SPME fiber phase were optimized based on the recovery of butyric acid. The addition of water resulted in the decrease in the recovery of the short chain fatty acids. A quantitative method was developed for butyric acid based on a calibration curve developed from direct liquid injections of standards into the GC.

INTRODUCTION

Off-flavor sugar directly affects the value of refined cane or beet sugar, which causes a problem to the sugar industry and consumers. The proposed quality control method involves using instrumental testing procedures to identify the off-flavors and determine and eliminate their sources (1). There are numerous compounds when present, even at trace levels, can render the sugar unacceptable. Olfactory sensory testing and quantitative results suggest that combinations of short-chain fatty acids are the most significant source of off-odors and off-flavors in sugar (2).

Three short-chain fatty acids in particular contribute to the overall flavor profile of off-flavor sugars: These are acetic, propanoic, and butyric acid (1). Hexanoic and 2-methyl-butanoic acid (isovaleric acid) can also contribute to off-flavor. Sugars with large amounts of these fatty acids have characteristic sour and rancid aromas and flavors (3). The presence of short chain fatty acids is a general indication of damage to the sugar as a result of improper handling or processing.

Sugar analysis for flavor quality control involves isolating and identifying trace amounts of compounds that contribute to off-flavors in products. Many studies have utilized solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) for analysis in a variety of fields, including food and agriculture industries (4). Despite the extensive use of this method, research on sugar has not been conducted using this technique. Therefore, SPME-GC-MS would appear to be an ideal candidate for the analysis of compounds which contribute to off-flavor aromas in sugar. This versatile technique could allow for the analysis of aromas in sugar in the form of crystals, powders, or syrups.

Solid-phase microextraction (SPME) was developed to identify the analytes that are present in the headspace or liquid phase of samples such as food, soil, or water (8,9). A block diagram of the procedure is shown in figure 1. This technique was originally intended for analysis of environmental samples but was quickly adopted by other industries, specifically the food and agriculture industries. SPME is often coupled with other techniques such as gas chromatography-mass spectrometry (GC-MS). This technology is simple, accurate, and efficient (6,7) and is suitable for qualitative analysis. Quantitative analysis using SPME is possible, yet problematic (8,9).

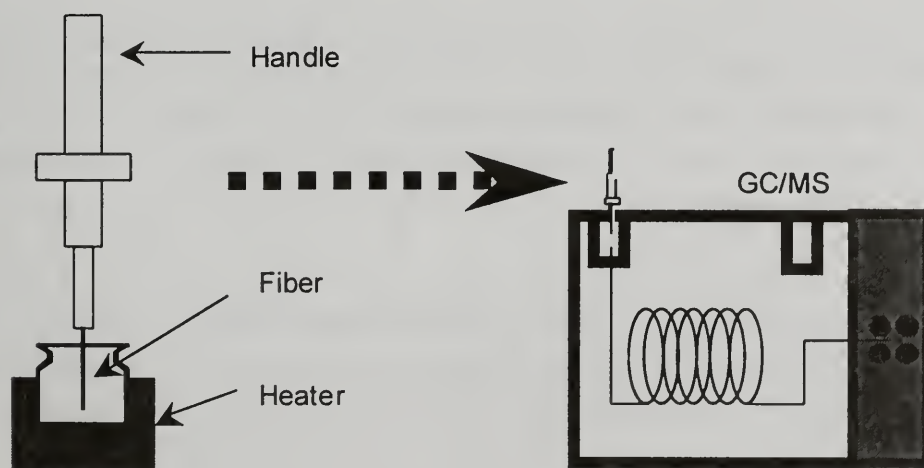


Figure 1. A block diagram of the SPME-GC-MS method.

MATERIALS AND METHODS

Analyses were performed using sugars provided by Sugar Processing Research Institute, Inc. through various proprietary industrial sources. 78 sugars, including raw cane sugar and white cane and beet sugar, were ranked based on butyric acid content and divided into three categories. Sugars remained at room temperature until sample preparation, which involved placing 0.75g of sugar into 2 mL vials. Milli-Q water (0-80 μ L) was added beneath the sugar with a syringe. Vials were sealed with a crimp cap fitted with a Teflon septum. Samples were run in triplicate and stored at room temperature (for up to 2 hours) until analyzed.

Samples were placed in a CTC SPME autosampler (Leap Technologies, Carrboro, NC) and individually heated in a sample agitator at 70°C for 15 minutes. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS - 50/30 μ m film) fiber was utilized during the 15-minute extraction period (Supelco, Bellefonte, PA). Sample volatiles were desorbed into the injection port of an Agilent 6890 GC equipped with a 5973 MS system (Agilent Technologies, Palo Alto, CA). Helium was utilized as the carrier gas under a constant flow of 36cm/s through a 30m, 0.25 μ m, DB-5 capillary column (J & W Scientific, Folsom, CA). The initial GC temperature (50°C) was held for 1 minute. The temperature was ramped first at 5°C/minute to 100°C, then at 15°C/minute to 270°C and held 5.67 minutes. Each 30 minute GC run was followed by a 5-minute cooling period.

The mass spectrometer was operated in scan mode. With the exception of propanoic acid, the base peak of the short-chain fatty acids was found at m/z 60. The integrated peak areas of m/z 60 were used for quantification of volatile fatty acids, and qualifier ions were examined with regard to retention time and ion ratios. The molecular ion and base peak for propanoic acid was found at m/z 74 and used for quantitation.

Compounds in the headspaces of the samples were tentatively identified using the Wiley mass spectral library (7th Edition). To determine the recovery of volatile short-chain fatty acids in the headspace, a single sugar sample was repeatedly analyzed until no analyte was observed. Samples were analyzed in triplicate to give standard deviations. To generate a calibration curve an authentic standard of butyric acid was obtained and a stock solution of 1 part per thousand was made in methanol. Standard dilutions of 0.1, 0.5, 1, 5, 10, 50, 100, 500, 1000 and 5000 ng/ μ L were prepared and one μ L injection of each dilution was made in triplicate. A calibration curve was developed based on the integrated peak area of m/z 60 as a function of mass of butyric acid injected. No response was observed for the 0.1 ng/ μ L injections and the 0.5 ng/ μ L injections were detectable but not quantifiable. A correlation of 0.9750 was found for the range of 1 ng/ μ L to 5000 ng/ μ L.

RESULTS AND DISCUSSION

SPME is an equilibration technique and dependent upon a number of sampling parameters including headspace volume, fiber stationary phase, temperature, mechanical mixing, and exposure time. For fatty acid analysis, extended adsorption periods generally result in greater loading especially for the longer chain fatty acids (10,11). In competitive cases where the fiber quickly becomes saturated, extended adsorption periods can actually result in a decrease in the adsorption of some compounds (12). Extended SPME adsorption periods are not practical when using an autosampler and running large numbers of samples. In these cases, the SPME adsorption period needs to be less than the GC run time and the optimal recovery may not be achieved. An adsorption period of 15 minutes was found to be adequate for short chain fatty acid analysis.

Polar molecules such as the short-chain fatty acids are generally detected more readily using a polar coating such as polyacrylate (12), whereas the analyses of non-polar molecules is accomplished using a non-polar coating such as PDMS. However, the DVB/CAR/PDMS fiber extracted the short-chain fatty acids more readily than the PA and PDMS fibers. Previous analysis of sugar volatiles employing thermal desorption, indicated that the addition of small amounts of water increased the detection of volatile compounds released from the sample (1). However, in SPME, the addition of water to the sample can suppress some volatile compounds while enhancing others (13).

Acceptable, borderline acceptable, and unacceptable sugars were analyzed in triplicate with a DVB/CAR/PDMS fiber, in the absence of water, at temperatures of 50°C, 60°C and 70°C. The error represented by the standard deviation of the “acceptable” sugar was lowest for the samples analyzed at 60°C (Table I). However, the integrated peak areas (m/z 60) for the “acceptable” sugar were all less than 6000 counts, which is barely above the baseline, thus the sample contained a negligible amount of butyric acid (< 1 ng). The standard deviation of the samples analyzed at 70°C was less than the standard deviation of the samples analyzed at 50°C and 60°C for both the “borderline unacceptable” and “unacceptable” sugars. Greater precision was observed at an adsorption temperature of 70°C.

Table 1. Average responses and relative standard deviations (RSD) of butyric acid in 3 white sugars at three temperatures.

	Acceptable Average RSD (%)		Borderline Average RSD (%)		Unacceptable Average RSD (%)	
50°C	1781	173	7286	35	21048	19
60°C	1506	25	9056	27	24336	48
70°C	2522	27	12609	11	29451	10

An unacceptable white sugar was analyzed in the absence of water with the DVB/CAR/PDMS fiber at an adsorption temperature of 70°C. A total ion chromatogram (TIC) is presented in Figure 2. Prominent peaks were identified based on the library searches of their mass spectra. The first-half of the chromatogram is dominated by the presence of the short-chain fatty acid peaks. Several siloxane contaminants routinely seen in SPME analysis are marked with an asterisk. Straight chain fatty acids (C_2 through C_9) were observed as well as three branched short-chain fatty acids (3-methyl butanoic acid, 2-methyl-butanoic acid, and 2-ethyl-hexanoic acid). The presence of some short-chain alcohols and aldehydes has been attributed to lipid oxidation and microbial activity. Short-chain fatty acids are not the sole source of off-flavor in sugar. However, their presence serves as a marker for an unacceptable product. A reconstructed ion chromatogram of m/z 60 is presented in Figure 3. With the exception of propanoic acid, the C_2 - C_9 acids are readily observed.

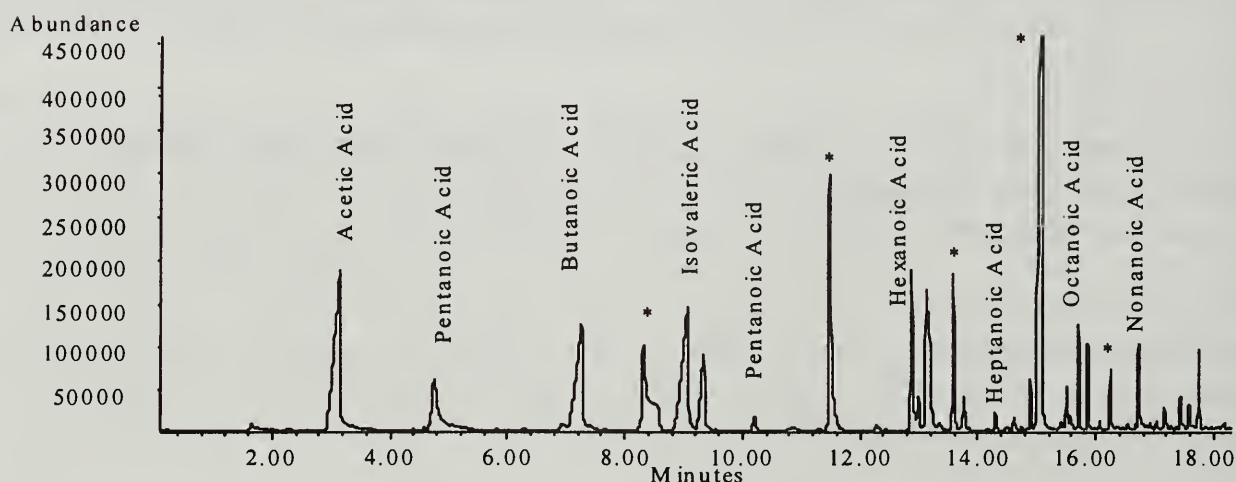


Figure 2. Total ion chromatogram (TIC) of compounds in an unacceptable white sugar. Peaks marked with an asterisk represent contaminants from the fiber/septa.

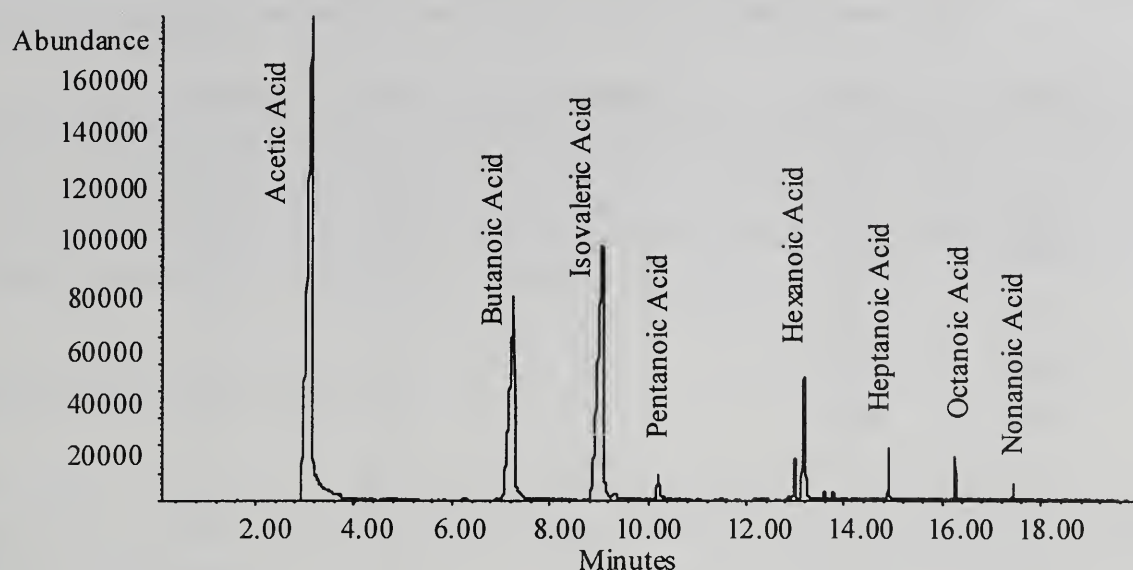


Figure 3. Reconstructed ion chromatogram (RIC) of identified short-chain fatty acids in an unacceptable white sugar (m/z 60).

For quantification using SPME, a calibration curve was generated using known amounts of butyric acid injected into the GC. Assuming that both the liquid injection and the SPME injection techniques are similar in efficiency, a plot of the integrated peak area against the 0.5 ppm (0.5 ng) standards were detectable but the signal to noise ratio was less than 2. This set the detection limit between 0.5 and 1 ng. The volatile and semi-volatile compounds in the headspace of the remaining 77 beet and cane sugar samples were analyzed by SPME-GC-MS. Table 2 lists the amount (ng) of butyric acid and the calculated concentration found in each of the sugars. The concentration is an estimate and serves as the minimum amount present. Values above 5000 ng have been extrapolated using the calibration curve and our tentative at best. Acceptable sugars tend to contain less than 75 ppb, while borderline sugars contain from 75 to 150 ppb, and unacceptable sugars contain greater than 150 ppb of butyric acid.

With the exception of propanoic acid, chromatographic peak areas were integrated based on m/z 60. Propanoic acid lacks a proton on the gamma carbon necessary for the McLafferty rearrangement and m/z 60 is not observed. The base peak and molecular ion at m/z 74 is used instead. Acetic acid also lacks a gamma carbon but readily forms a molecular ion as the base peak at m/z 60. Acetic acid is the most abundant short-chain fatty acid. A general trend of decreasing concentration is observed with increasing chain length. This may well be an artifact due to the SPME adsorption process with the more volatile compounds being recovered more efficiently.

**Table 2. Amount of butyric acid detected in 78 sugar samples,
with concentration based on a recovery value of 38%.**

Sugar No.(ng)		(ppb)	Sugar No.		(ng)	(ppb)
0	13	18	39	117	156	
1	5488	7317	40	134	178	
2	10828	14438	41	30	40	
3	167	222	42	31	42	
4	177	236	43	52	70	
5	6846	9129	44	110	147	
6	6402	8536	45	103	137	
7	33	44	46	55	74	
8	32	43	47	172	229	
9	22	29	48	35	46	
10	24	32	49	123	164	
11	18	24	50	94	126	
12	18	23	51	63	84	
13	23	30	52	5	6	
14	127	169	53	30	40	
15	19	26	54	186	248	
16	49	66	55	74	98	
17	277	369	56	194	259	
18	67	90	57	367	490	
19	14	18	58	200	267	
20	92	123	59	91	122	
21	5	7	60	273	365	
22	13	18	61	14	19	
23	107	143	62	17	23	
24	96	128	63	5	6	
25	98	131	64	5	6	
26	12	16	65	6	8	
27	23	30	66	5	6	
28	154	201	67	5	6	
29	87	116	68	5	6	
30	242	323	69	139	186	
31	104	139	70	10	13	
32	100	133	71	12	16	
33	168	224	72	16	21	
34	80	107	73	57	76	
35	31	41	74	23	30	
36	44	58	75	70	93	
37	122	163	76	25	33	
38	149	199	77	20	27	

CONCLUSION

Employing SPME-GC-MS, the short chain fatty acids series from acetic to nonanoic acid was observed along with the branched chain fatty acids, 3-methyl butanoic acid, 2-methyl-butanoic acid, and 2-ethyl-hexanoic. A calibration curve based on injected standards was used to provide semi-quantitative information for comparing relative amounts of butyric acid between samples.

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APPLICATION OF HOP BETA ACIDS AND ROSIN ACIDS IN THE SUGAR INDUSTRY

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ABSTRACT

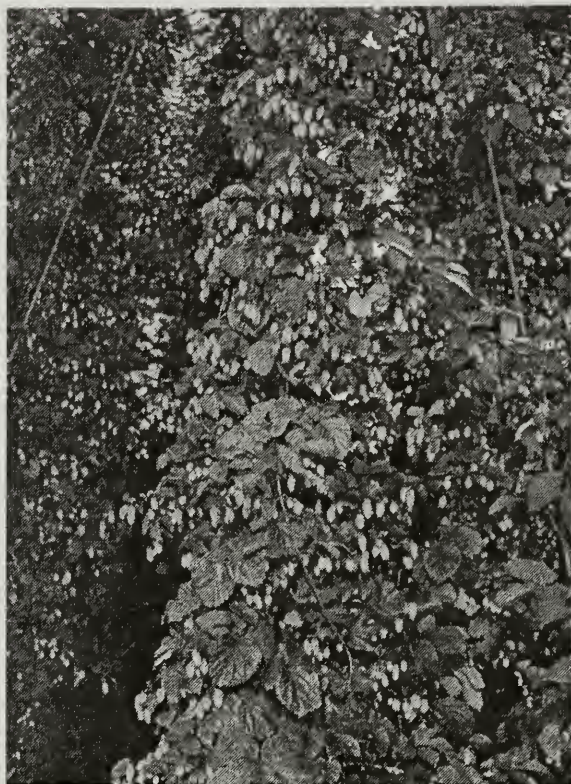
In 1994 hop products were successfully used for the first time to combat bacteria in beet extraction. This was a totally new field for the use of hop products, compared with their traditional use in breweries. Today an alkaline solution of hop beta acids is used in sugar factories under the trademark "BetaStab". Hop beta-acids have turned out to be very effective against formation of NO_2 and anaerobic infections in tower extractors, which are often operated intentionally with lactic acid fermentation. Hop beta-acids have additionally proved effective in the field of thick juice storage. Sometimes, in the case of lactic acid control, a selection of less sensitive organisms is observed and a second disinfectant has to be used, alternating with hops. Thus an idea was born to use rosin acids as a further harmless natural biocide. Results from laboratory trials, full scale trials and some first studies on residues are presented. Rosin acids show a potential to be used in the sugar industry, either alternating with hop products or to create products which are more cost effective.

INTRODUCTION

In 1994 the first experiments with hop beta acids were carried out at the Agrana factory in Tulln, to control bacteria in beet extraction [1-2]. Agrana voluntarily abstained from using formalin and dithiocarbamates in sugar factories in 1991, when formalin hit the headlines due to emissions from chipboards. It was not always possible to eliminate bacterial problems simply by using higher extraction temperatures, so the idea to use hop beta acids in sugar factories occurred at the right moment. These natural water-insoluble hop-components are considered harmless for human beings and mammals and are regularly consumed by people favoring turbid white beer. By now, these beta acids have already been used in the sugar industry for as long as 8 years. Compared with an 800-year tradition of hopped

beer, obviously hop acids in the sugar industry do not have an excessively long history, but at least some international experience already exists.

Hop plant



Pine tree



Figure 1. New useful plants for the sugar industry.

On the other hand, the use of rosin acids for bacterial control in the sugar industry is quite a new idea without any international experience. We would like to take the opportunity to present this topic and some of our own results at this conference for the first time. Rosin acids from pine trees (Figure 1) would fit well into a range of natural products for bacterial control and the original idea was derived from the Greek „Retsina“ wine, produced with pine gum from the Aleppo-pine. The pine gum is added during fermentation and is effective against oxidation and acetic acid production under hot climate conditions [3]. After 2000 years of tradition, this Retsina wine with its characteristic taste even tops the long history of hopped beer. Fortunately, the non-volatile, water-insoluble fraction of pine gum, the so-called “rosin acids” (or “resin acids”), show a similar bacteriostatic effect to hop beta acids, although at higher concentrations. We preferred to start work with these rosin acids, to be sure that white sugar will not show any characteristic odor of turpentine oil, the volatile fraction of pine gum.

Retrospective view on hop application in the sugar industry

Most of the scientific papers dealing with the mode of action of hop acids on bacteria were published within brewing science. *Shimwell* [4] found a parallelism between bacterial sensitivity against hop-acids and staining properties and also the great importance of pH on the effect. Gram-positive bacteria are affected by hop acids, but not spores and Gram-negative bacteria, although there are some exceptions [5]. Fortunately, most of the known bacterial species, growing in hot juices of beet sugar factories, are Gram-positive or at least sensitive against hop beta acids. In recent decades, scientific papers explained the effect of hop acids on bacteria as a damaging effect on the function of the bacterial membrane [6] and as lowering of the intracellular pH [7]. A more detailed quotation of papers outside the sugar industry was given in the first paper about hop application in the sugar industry [2].

After the first paper on hop application, eight further lectures or posters have been presented at conferences or meetings, where at least summaries have been published [8-15]. Unpublished information from BetaStab customers is regarded as confidential. In the earlier papers a so-called "Baseextract" was used in emulsified form [2][8-9], containing roughly 50 % beta acids, the least soluble but most effective hop acid. Later on beta acids were isolated and offered as clear, well-defined and even more effective alkaline solution, which today is well-known as "BetaStab 10A" or simply "BetaStab".

Practical experience can be drawn from reviewing papers with different conditions rather than from a lot of repetitions in one country. Lowering of lactic acid levels is priority in factories without severe microbial problems, in order to reduce losses. As lactic acid production is possible for a lot of bacterial species, it is imaginable that less sensitive organisms occur, which cannot be controlled by minimal inhibitory concentrations, but only by shocks with high concentrations.

"Sterile" beet extraction with lactic acid suppression

A very good result for a DDS extractor was reached in 1998 in an Italian factory [12], operated with 250 ppm of SO₂ on beet. For this trial BetaStab emulsion was still used. The emulsion was poured into presswater, port 2 and port 3, dependent on individual lactic acid and NO₂ determination. This led to a ten days average of 68 ppm of lactic acid in raw juice, which was very close to the beet values. If a tower extraction is kept as "sterile" as possible, shock application to, at least, presswater, mid-tower position and to cossette/juice pump are required (Figure 2). Dosing at mid-tower requires an especially good distribution between the outside wall and central cylinder, while the distribution around the periphery will arise from rotation.

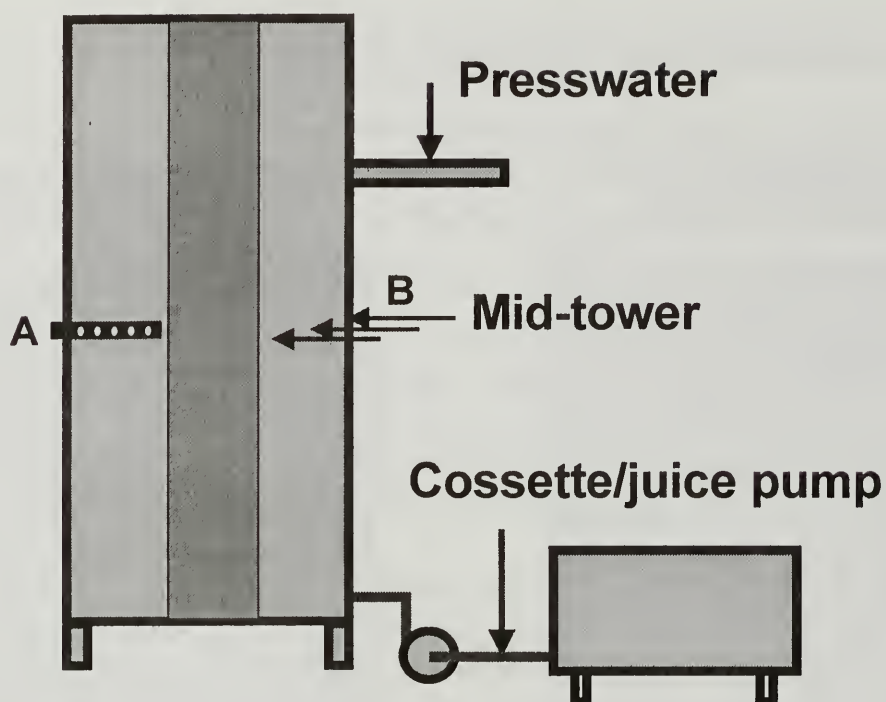


Figure 2. “Sterile” beet extraction with lactic acid suppression.

Tubes with several holes, leading from the wall close to the central cylinder and shown as variant A, were often installed at the time of tower erection for dosing of formalin. If shocks with lower volumes of BetaStab are not heavy enough, the inner holes will not be served and biofilms on the inner part of the tower will not be affected. Continuous dilution of BetaStab with water during shocks may improve the distribution. In Austria we even have three tubes, shown as variant B, with different lengths, which are served alternatively by valves in periods of 20 seconds.

During the first days BetaStab is often very effective with a single dosing point at the mixer, because traces of beta acids are carried upwards with the cossettes and dissolved in the juice. The minimal inhibitory concentration for bacteria, which are not able to grow in presence of hop beta acids, will be exceeded in favorable cases. If the effect diminishes, the existing know-how about dosing and shocks will be important. Installations can be improved after the campaign, but usually not during trials. If the knowledge about effective dosing does not help, a rotating use of two different biocides may do. For example, a combination of BetaStab and ABS (ammonium-bi-sulfite or -hydrogen-sulfite) has been used in such a way, as reported by *M. Fowers* [14].

Beet extraction with intentional lactic acid fermentation

More and more European sugar factories use micro-organisms in extraction towers to improve pulp pressing. A so-called homo-fermentative lactic acid fermentation without gas formation would be welcomed in the upper part of the tower (Figure 3).

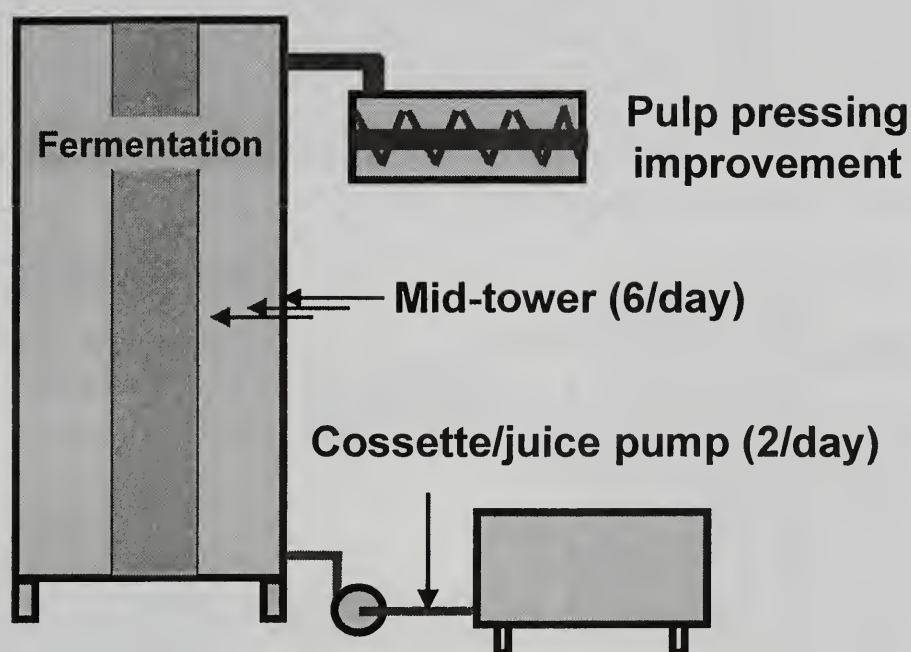


Figure 3. Beet extraction with intentional lactic acid fermentation.

Therefore no biocides are added to this upper part. A certain amount of lactic acid, formed in this part, will appear in the raw juice and has to be accepted. Normally Austrian factories are satisfied with a maximum of 400 mg/L of lactic acid in the raw juice. According to Austrian findings, six shocks a day at mid-tower position and fewer shocks at the cossette/juice-pump are used to limit excessive lactic acid formation. As the upper part of the tower acts as inoculum for the lower part, it is not always possible to get an economic lowering of lactic acid.

Lactic acid fermentation and misfermentation

In contrast to the Italian DDS extraction, already mentioned, we nearly failed in lowering lactic acid in a Czech DDS trough, operated with sulfuric acid for supply water [8]. Additionally the presswater temperature was low and a lot of D-lactic acid was produced, indicating a growth of *Lactobacilli*. But we learned a lot about misfermentation and specific effects of hop acids on the occasion of this failure.

Acetic acid and NO_2 dropped in parallel to very low levels and in an earlier paper we were able to show that they are connected to each other [11]. Now we have found a pathway, reported for *Escherichia coli*, which shows a production of acetic acid and CO_2 as a consequence of NO_2 formation [16-17] and fits well to our observation.

In contrast to lactic acid, NO_2 is completely unwanted in the sugar industry and thus is a product of misfermentation, which can be avoided by hop addition. A further degradation of NO_2 leads to a heavy gas formation, as already shown by Carruthers et al. [18]. The intensity will be dependant on the properties of the actual factory flora, growing in lower

parts of the tower and in the mixer. This gas formation will be inhibited by blocking the formation of NO_2 with BetaStab. German reports [13] about an economic drop in antifoam consumption on BetaStab addition could be explained by this mechanism. But this is not definite, because a second type of gas forming infection occurs in extraction towers, which does not use nitrate: It is the hydrogen producing Clostridia-type (Figure 4), with the new taxonomic designation "*Thermoanaerobacter(ium)*" [19-20].

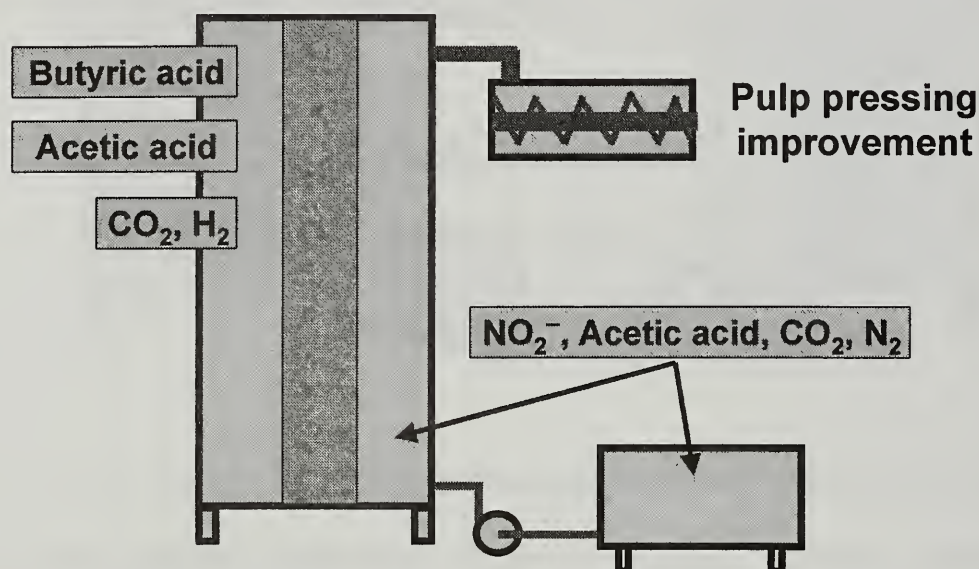


Figure 4. Lactic acid fermentation and misfermentation.

As the good effect against NO_2 was connected to acetic acid production, we put the question if hop products could suppress other acetic acid, stemming from these Clostridia, in the same way. If an important pathway of Clostridia is blocked, their growth and all these unwanted acids and gases would disappear. Indeed it was possible to convert a fermentation, caused by Clostridia, to a lactic acid fermentation [11]. Low dosing rates or shocks are necessary to maintain the lactic acid fermentation.

The well-known selection of alcoholic fermentation by yeasts over other fermentation was the original purpose of hops for beer and pine gum for wine. This is comparable to a selection of lactic acid fermentation over an unfavorable misfermentation in the sugar industry. Normally no drop in effect will appear because of a common effect of hop beta acids and lactic acid formation. It is impossible to get a similar effect with formalin, which is one of the least selective biocides [21].

Effects under slight alkaline conditions

Under slight alkaline conditions hop beta acids are more dissociated and thus should be less effective. But they are more soluble and have shown surprising effects on control of *Thermus* in thin juice, which passed an ion exchange column and lead to increase of NO_2 (Figure 5) [2][5].

This positive effect under slight alkaline conditions was encouraging to start trials in another field of slight alkaline conditions: Thick juice storage (Figure 5). BetaStab was a little more effective against invert sugar formation than against a pH drop [15]. As a detectable invert sugar formation is an extracellular reaction, the question arises if the mechanism of hop beta acids is particularly effective against excretion of enzymes by bacterial cells.

Control of Thermus sp.



Thick juice storage trial

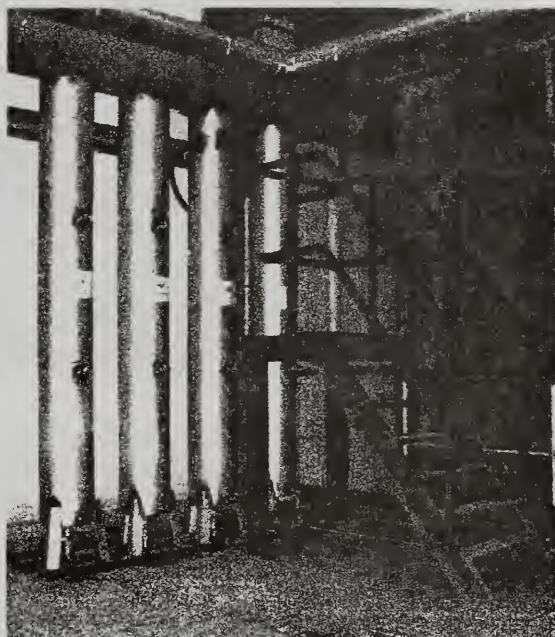


Figure 5. Effects under slight alkaline conditions.

Problems with Dextran in a Czech factory

There is a very important excretion of a bacterial enzyme for the sugar industry, which appears in raw juice as well as under slight alkaline conditions of preliming: It is the Dextran-forming Hexosyl-transferase from *Leuconostoc*. In a Czech factory problems with Dextran, stemming from this organism, occurred in spite of routine disinfection with formalin and other biocides [8], but disappeared after application of BetaStab. As manual work was necessary to remove Dextran pellets (Figure 6), BetaStab was very welcomed.

Till now we have two observations from two campaigns, but the problems did not come back after we stopped dosing. As a return of infection is important to get repeated blanks, we cannot show analytical results from these full scale trials in the experimental part. But for completion of the review and with respect to the importance for cane the observations about polysaccharide inhibition should be mentioned and a preliminary laboratory trial will be shown in the experimental part.

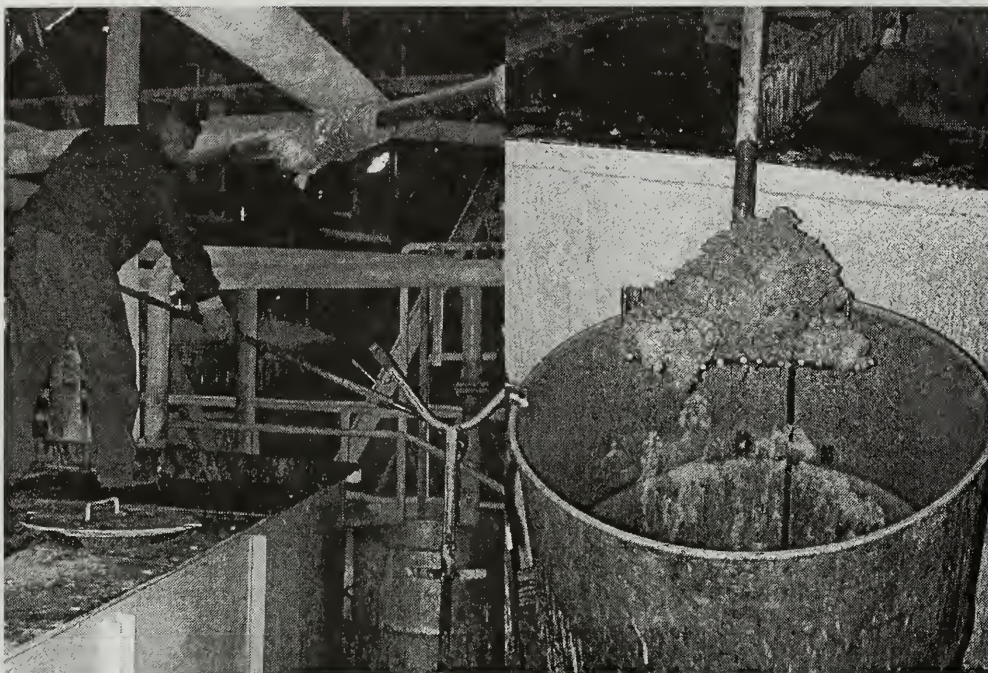


Figure 6: Problems with Dextran in a Czech factory.

INFORMATION ON ROSIN AND ROSIN ACIDS

Pine gum components

After this review on hop application the paper proceeds with the presentation of rosin acids. Of course, a lot of progress is still necessary to put this new idea into practice and possible handicaps should be found out as soon as possible. We hope that the "family of hop-users", who may have benefited from this new variant of natural biocide, will contribute in the same way as for hop application in the past, and thus will be mentioned and quoted in a rosin review one day.

In contrast to many new developed chemical biocides, a mixture of rosin acids is already well-known, but not for use in the sugar industry. Gum products from the pine tree are distilled into about 20% volatile and 80% non-volatile components. The volatile fraction is called "turpentine oil". The mixture of rosin acids, as visible on the right, is called rosin or often "colophony" (Figure 7), and this popular name should be at least mentioned here.

Turpentine oil, containing the volatile fraction of pine gum with the main components α -Pinene and β -Pinene (Figure 8), is harmful to the skin and if swallowed neat, and is included to the European Union Biocide List. As already mentioned, we did not want to use it for sugar factories to avoid its strong characteristic odor. On the other hand, the particular effect of abietic acid (Figure 8) and their isomers (Figure 9) against thermophiles is not known in the food industry.

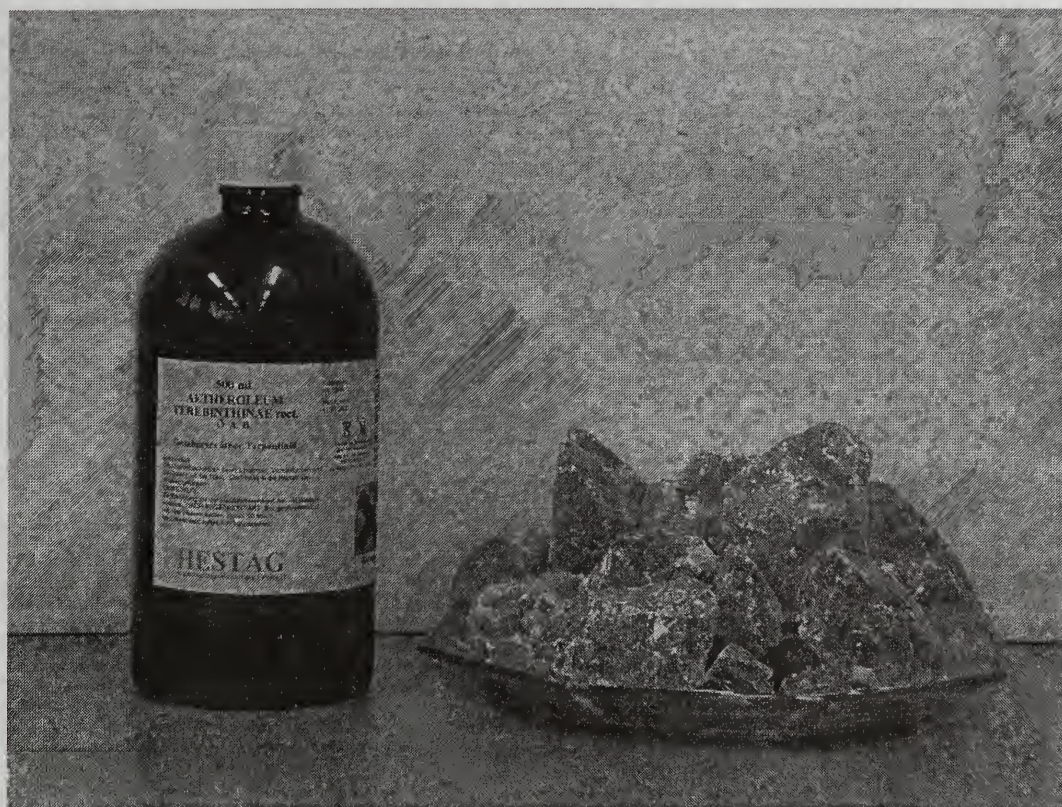


Figure 7. Volatile and non-volatile pine gum fractions: turpentine oil and colophony.

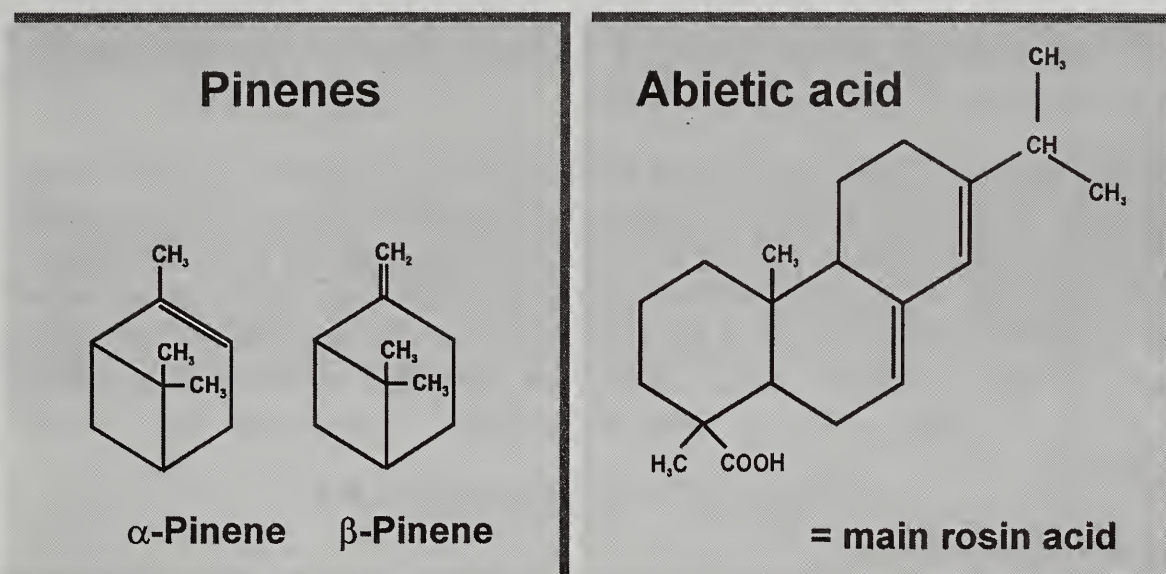


Figure 8. Volatile and non-volatile pine gum components.

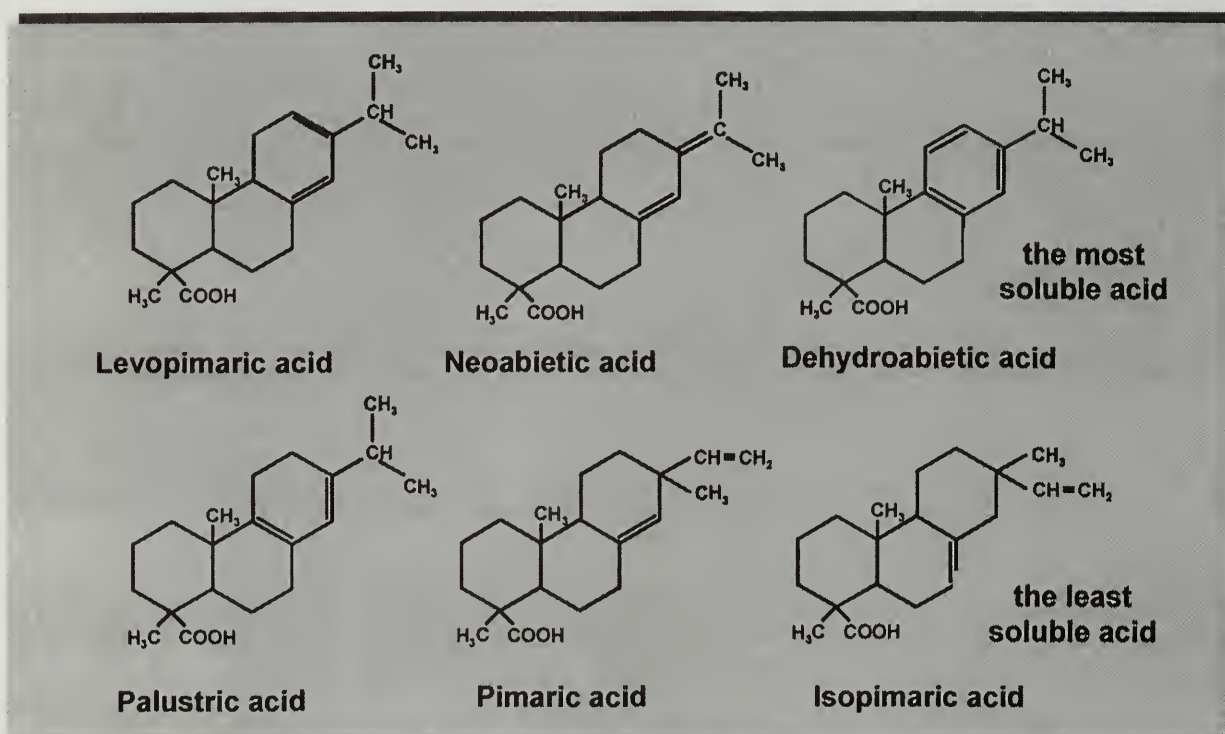


Figure 9. Abietic acid isomers and Dehydroabietic acid.

We found a patent which shows minimum inhibitory concentrations of 20 ppm for abietic acid against Acne bacterium [22]. In the field of food technology no information could be found and therefore patents have been filed [23-24].

We did not find scientific papers, comparable to brewing science, which explain the effect of rosin acids on bacteria. But scientific papers deal with properties of rosin acids, emitted to wastewater from paper mills, as they are toxic for fish. It is stated that isopimaric acid is the most toxic and least soluble acid, whilst dehydroabietic acid is the least toxic and most soluble one (Figure 9) [25]. This shows a parallel behavior to hop products and their effect against bacteria, although different contact surfaces are influenced. More papers have been published about degradation of rosin acids by bacteria, demonstrating their natural character [26-28].

Toxicology of rosin products

Indeed, the statement shown as Figure 10 about toxicology of rosin products [29] doesn't show much danger for rosin acids. "Harmless" and "nontoxic", even "beneficial" are used as attributes. Besides Retsina wine, chewing gum is mentioned as a foodstuff and people will extract rosin acid derivatives and traces of rosin acids on chewing. Additionally, although not mentioned here, barrels for beer were covered inside with brewer's pitch, consisting of paraffin and rosin acids [30]. Long term studies of more than 25 years did not show health risks. However, rosin dust may be an allergic risk for some people.

Ullmann, 1993, Volume A23, Page 87:

„Native rosin can be regarded as harmless and nontoxic. This also applies to many of its derivatives and modified rosins. Rosin esters are used, for example, in chewing gum, and rosin wine (retsina) is considered to be particularly beneficial.

About a hundred workers, who were in daily contact with a large number of modified rosins, have been checked over a period of more than 25 years. No damaging effects on health could be established.

Allergic reactions of certain people cannot, however, be ruled out. There is no evidence that allergy initiation can be attributed to any toxicological properties of rosin.“

Figure 10. Toxicology of rosin products.

In the sugar industry rosin acids will be used as alkaline solution, similar to BetaStab. Thus allergy risks from dust will be kept away from sugar factories and considering all the other statements, a certification process for the sugar industry should not meet with big difficulties.

Until now we have only poor comparable examples for beet pulp as animal feed, as cattle do not really use chewing gum, although it looks like this during ruminating. But as rosin acids are harmless for human beings, traces in pulp may be harmless for animals, compared to traces of chemical biocides which are even active at stomach temperature. Even a beneficial effect could be possible, as reported for retsina wine and human beings (Figure 10) [29] or for larch tree extracts and pigs [31].

EXPERIMENTAL SECTION

MATERIALS AND METHODS

Detailed information about materials and methods is given in the appendix.

The laboratory trials were carried out in sterile glass vessels with pH recording and slow magnetic stirring, in order to avoid artificial aeration and to simulate anaerobic conditions in extraction towers. In addition to the most important information from pH-recording (Figure 11), samples were drawn for manual measurement of optical density and for

bacterial staining. The laboratory trials were carried out with clear liquid nutrients as usual in microbiology, inoculated with pure strains or simply with raw juice.

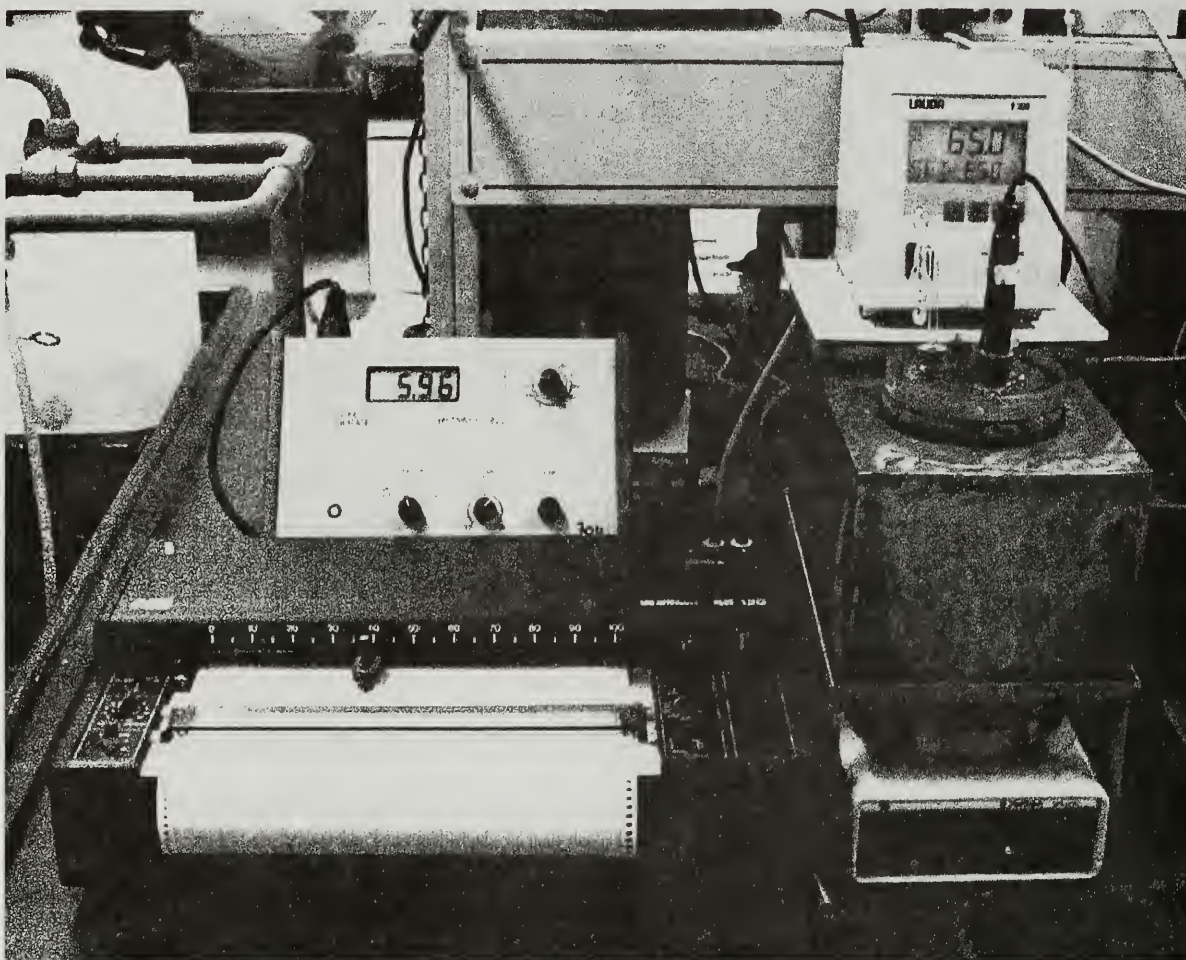


Figure 11. Equipment for laboratory trials.

Parallel to these laboratory trials full scale trials have been carried out, with measurement of relevant parameters, such as lactic acid and NO_2 . Considering our resources and the problems in Austrian factories, no polysaccharides have been determined till now.

RESULTS

Results from laboratory trials

Typical results from trials with pH-recording and raw juice as inoculum are shown in Figure 12. After two additions of 1 ppm of beta acids, the pH drop stops immediately and the curve is stable for about two hours. *Teuber and Schmalreck* [6] have used minimum inhibitory concentrations, abbreviated MIC values, for studies on the inhibitory mechanism of hop acids, defined as inhibition during at least one hour at 37°C . The inhibition at 65°C fulfills this time condition.

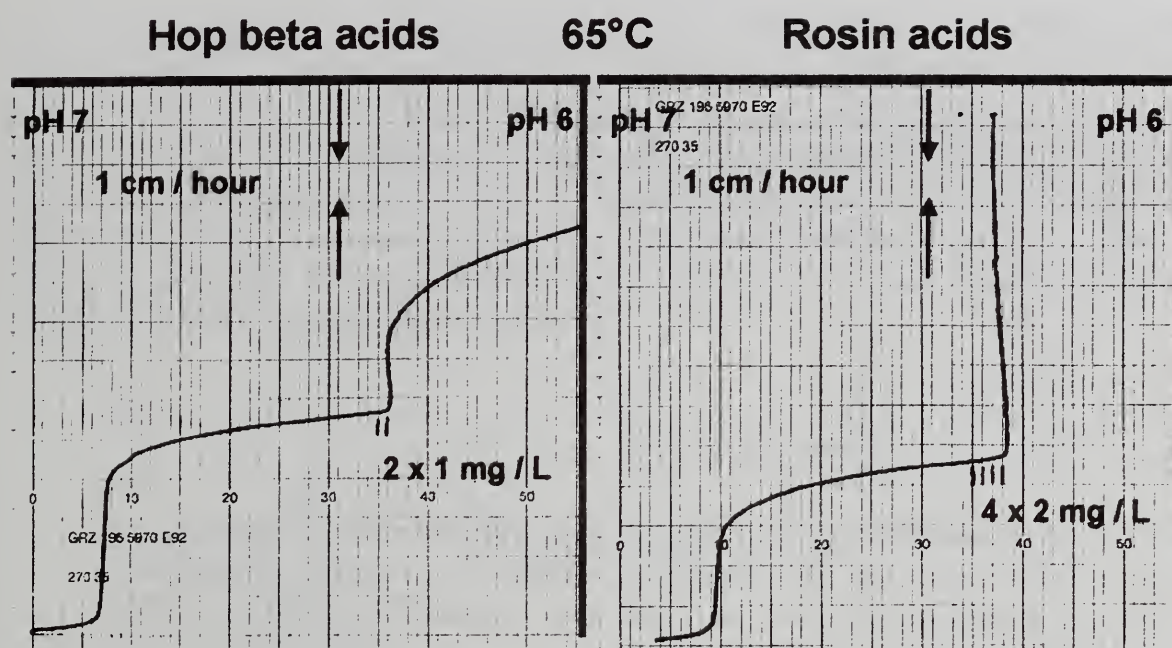


Figure 12. Comparison between hop beta acids and rosin acids.

It is typical for hop products and this experimental model, that MIC values are only effective for some hours and a further drop in pH will appear. Therefore other sugar scientists may be disappointed, if they want to see an effect from overnight laboratory trials without recording. But in sugar factories shocks with high local concentration, differing significantly from MIC values, are applied every four hours and effects are obtained, in spite of this further drop in pH.

Normally we shut off the trials by a timer after some hours during the evening, when the pen is already outside the scale. We have been surprised that in the case of rosin acids the trials were shut off by the timer during an interesting period. For rosin acids higher MIC values are necessary to get a first sharp stop in pH drop, but no further drop appears, even in overnight trials. A parallel behavior was found in full scale trials, which are shown later.

Table 1 shows MIC values, obtained by the same method, for single rosin acids. The results are in good agreement with statements from an already mentioned paper, dealing with toxicity for fish [25]. Dehydroabietic acid, the most soluble acid, is least toxic. Isopimaric acid, the least soluble, is one of the most toxic acids. Of course the absolute values are dependent on temperature, strain and pH, but the relative distribution is in agreement.

Table 1. MIC-values for strain DSM 457
"Titration"-method at 65°C

<i>Rosin acid</i>	<i>MIC-T65</i>	<i>Remarks</i>
Dehydroabietic acid	12	most soluble acid
Abietic acid	8	main rosin acid
Neoabietic acid	8	
Pimaric acid	8	
Palustric acid	8	
Levopimaric acid	6	
Isopimaric acid	6	least soluble acid

Figure 13 shows an important difference between hop beta acids and rosin acids on the one hand and formalin on the other hand. For formalin it would be impossible to determine a minimal inhibitory concentration by titration. After addition of neutralized formalin at 35 % of the recorder scale, comparable to the hop trial, the pH drop was inhibited slowly after some time, showing a curve instead of an angle. As formalin denatures proteins [21], the mechanism is quite different to membrane leakage, as reported for hop beta acids [6]. But after the slow inhibition of the pH drop no further drop appears during overnight. Glutaraldehyde shows a similar curve, while dithiocarbamates show an almost right angle, similar to hop beta acids and rosin acids.

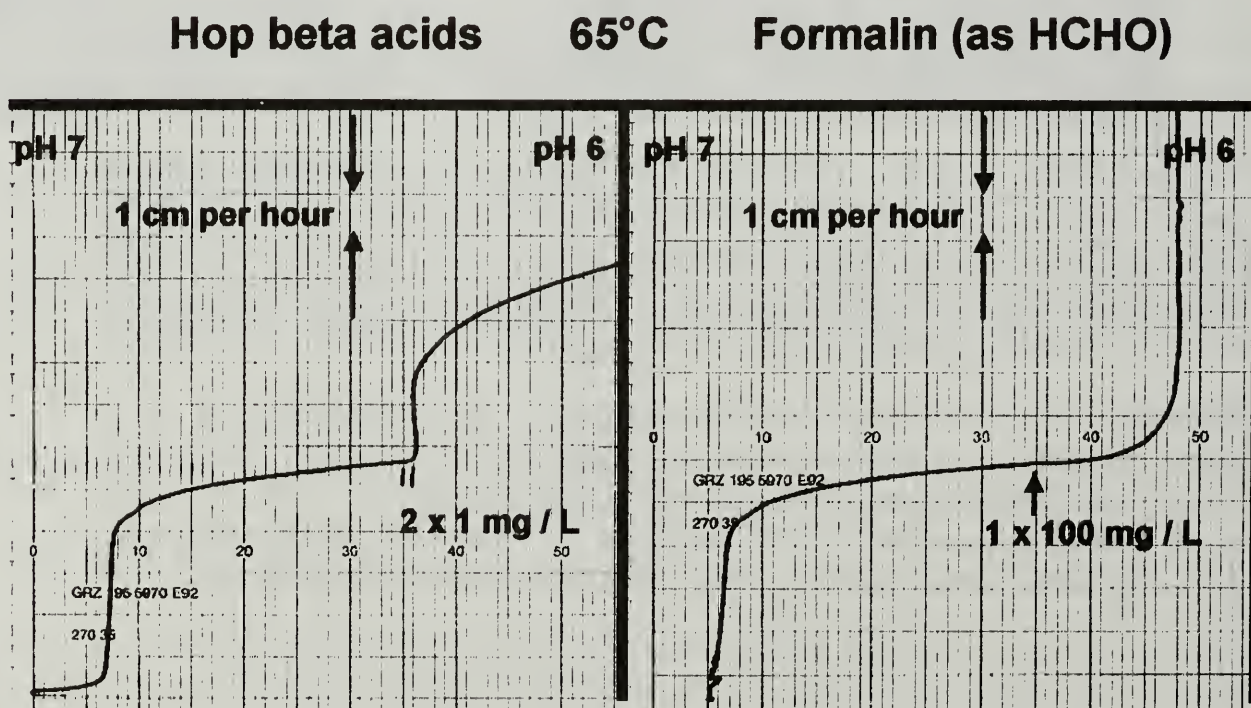


Figure 13. Comparison between hop beta acids and formalin.

It is usual to follow up microbial growth in clear artificial nutrients by measuring optical density at 600 nm, abbreviated as OD₆₀₀. We wanted to show the influence of both hop beta acids and rosin acids on optical density, measured against clear culture medium. Six ppm of rosin acids were necessary to stop the drop in pH and the increase of optical density (Figure 14). It was surprising that the optical density dropped back heavy and immediately after the addition. As optical density was measured by manual sampling, these values ended up after eight hours and only the pH was further recorded, showing a constant pH until the trial was switched off by the timer.

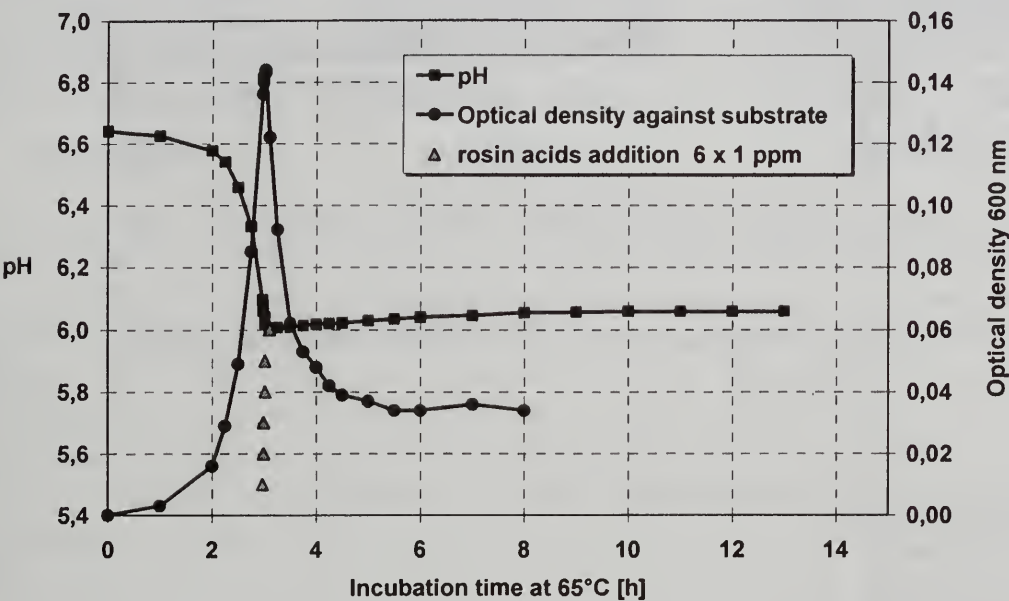


Figure 14. Influence of rosin acids on pH and OD₆₀₀ (pure strain DSM 2027).

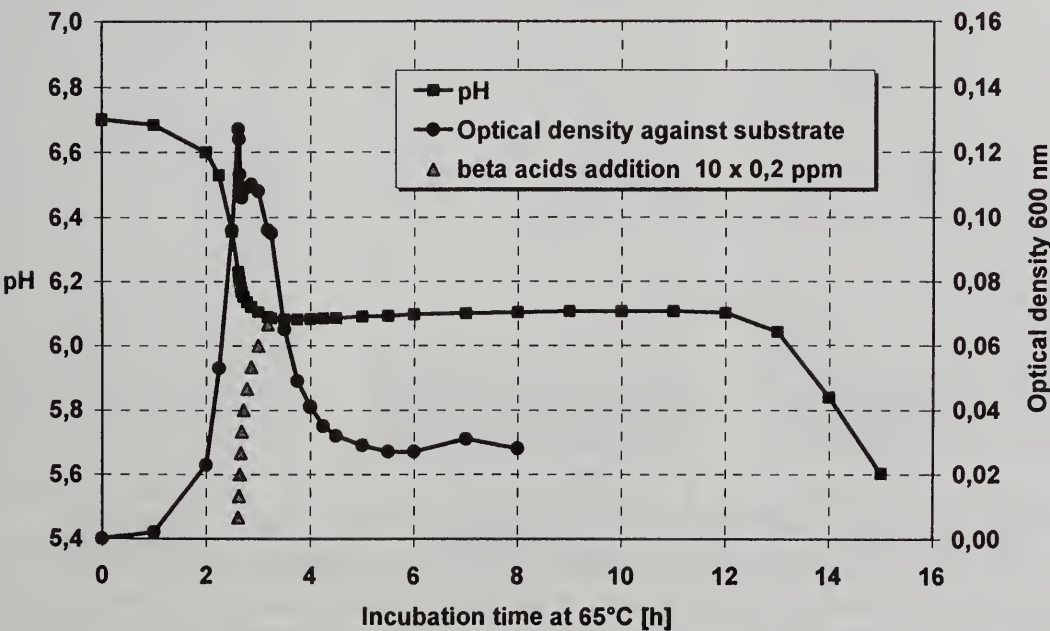


Figure 15. Influence of hop beta acids on pH and OD₆₀₀ (pure strain DSM 2027).

With hop beta acids the same effect occurred in optical density measurements (Figure 15). Ten small steps of addition led to a more rounded pH curve in this trial, but the optical density dropped heavily during this time. After several hours a second pH drop is visible in this culture with a pure strain. The same difference between hop beta acids and rosin acid was already shown with a raw juice culture and was obtained with a further strain DSM 22. We are not prepared to explain what happens in the bacterial cell with hop beta acids, but are only able to demonstrate the difference between hop beta acids and rosin acids. Thermophiles are reported to be extremely flexible organisms [32-33] and therefore an adaptation of strains could appear in the case of hop beta acids. Furthermore, these acids are more insoluble than rosin acids and could precipitate to an equilibrium below the MIC value of the original or the adapted strain.

The same drop in optical density appears with different inocula, either pure strains or simply 20 mL of frozen raw juice from a sugar factory (Figure 16). The MIC value is dependent on the type of inoculum. For raw juice from the Tulln factory, used as inoculum, the highest concentration of 8 ppm was necessary to stop an increase in optical density.

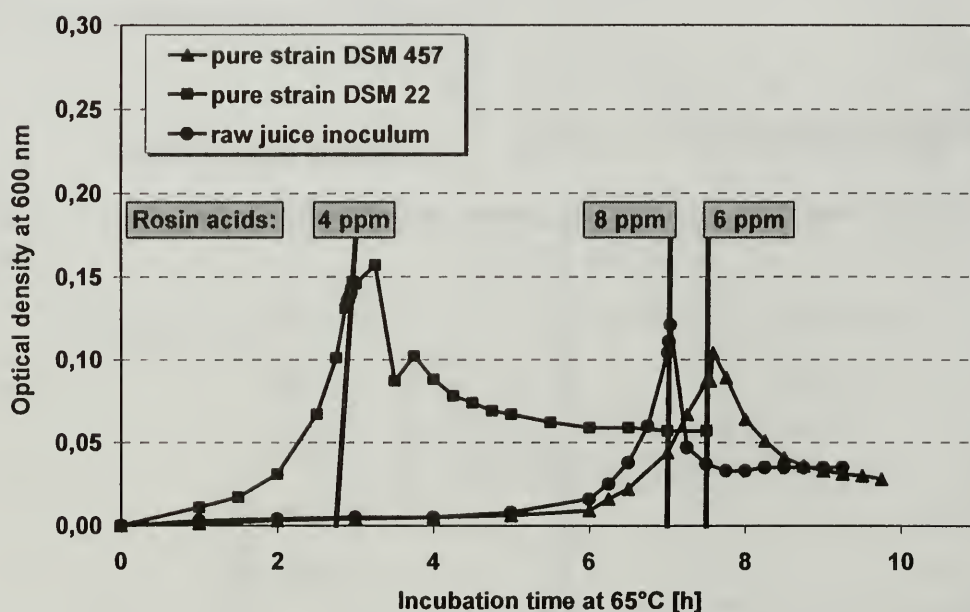


Figure 16. Development of OD₆₀₀ at 65°C with different inocula.

The congo red method is a vital staining method, introduced to the sugar industry by *Weidenhagen et al.* [34]. Living cells are able to resist to the colour (which changes from red to blue during preparation) and look white on a blue background. Dead cells are blue on blue and it is difficult to make them out. Shortly after rosin acid addition, the cells showed a lower contrast and after 22 minutes no cells were visible with the same method of preparation (Figure 17).

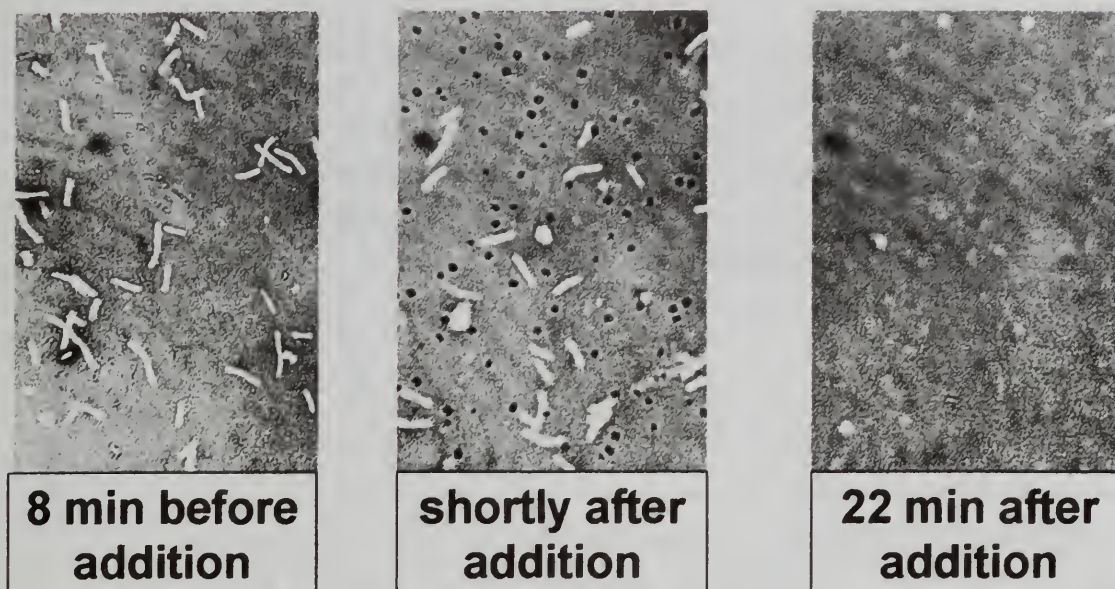


Figure 17. Congo red/blue staining of pure strain DSM 457 with rosin acids addition.

In a trial with raw juice inoculation cells with good contrast disappeared after two minutes (Figure 18). Considering our resources, it was impossible to analyze all possible combinations of hop beta acids, pure strains and methods. Therefore the new rosin acids have been put to the foreground and parallel conclusions may be drawn: The drop in optical density is caused by a dissolution of thermophiles at their growing temperatures, if the metabolism is blocked by hop beta acids or rosin acids. It is not simply a bacteriostatic effect with continuous growth after rinsing out of the “thermo”-biocides. Of course, spores are not effected and will be able to germinate within some hours after a biocide shock.

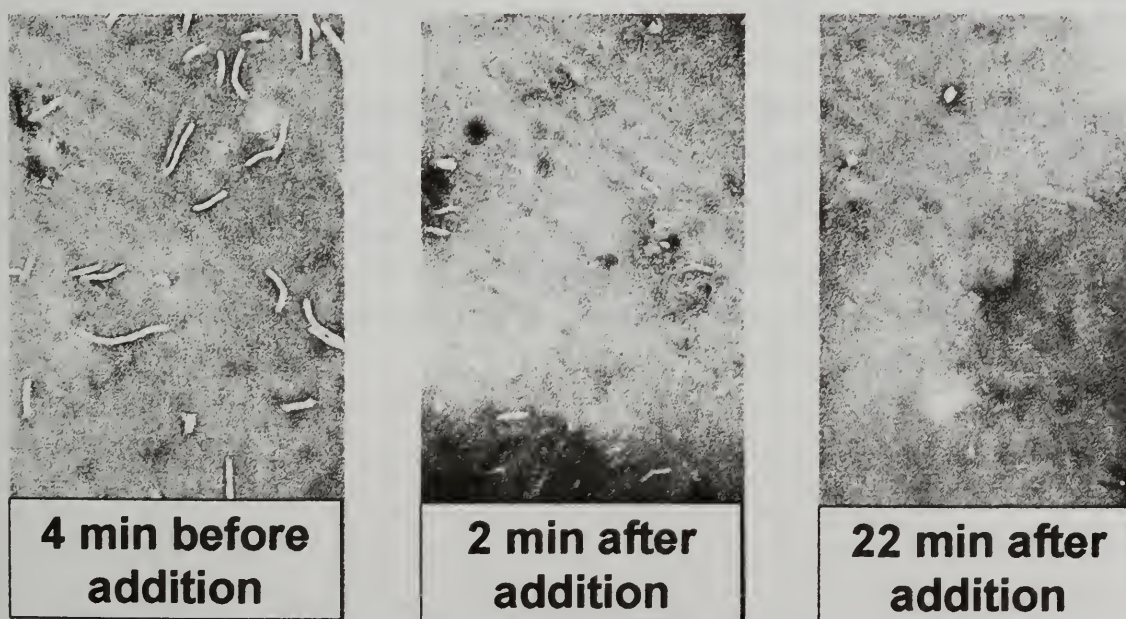


Figure 18. Congo red/blue staining of raw juice inoculated culture with rosin acids addition.

To demonstrate the particular effect of the natural acids at high temperatures, the optical density was measured for cultures of different temperatures (Figure 19). All trials were inoculated with 20 mL of raw juice. In contrast to the high temperature variant, it was impossible to stop cultures at lower temperatures with useful amounts of rosin acids and the addition was stopped at a very high level. Additionally, it was impossible to stop a drop in pH with both, hop beta acids and rosin acids, at temperatures of 40 and 35°C in further trials.

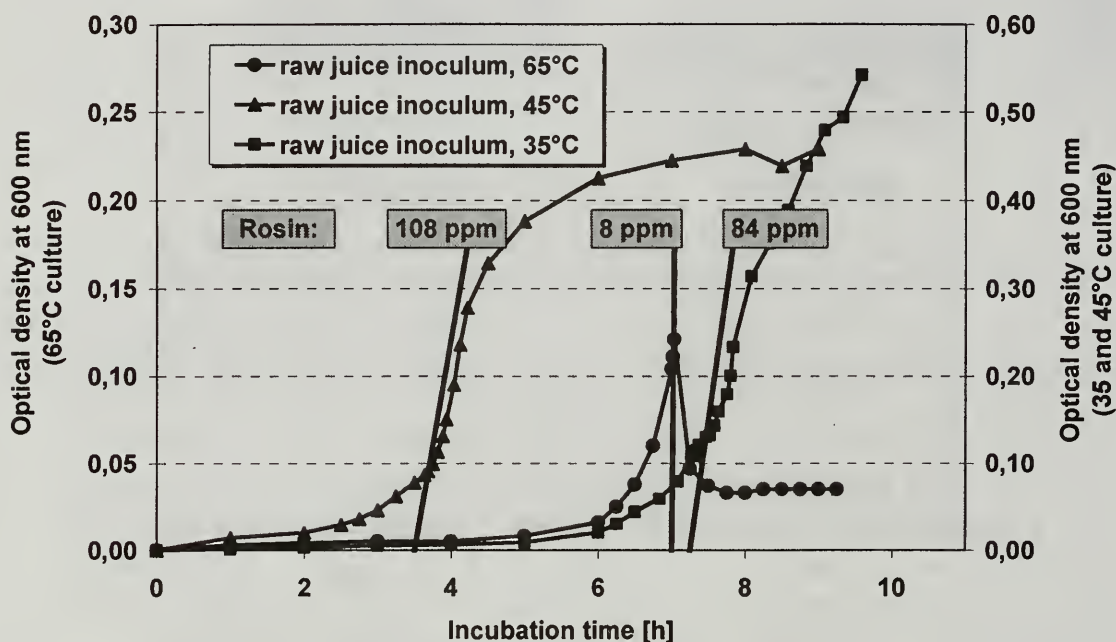


Figure 19. Development of OD₆₀₀ at different temperatures with rosin acids additions.

Leuconostoc is a mesophilic organism and we were not able to stop a pure strain under these anaerobic conditions. But under aerobic conditions, in shake cultures, an effect was obtained, with hop beta acids rather than with rosin acids (Figure 20). The concentrations are higher than those with thermophilic organisms and will only be reached during shocks and not on continuous dosing. This is typically a matter of further studies and not completed to date, as we have to focus our work on problems of Austrian factories, such as lowering of lactic acid and NO₂ from towers.

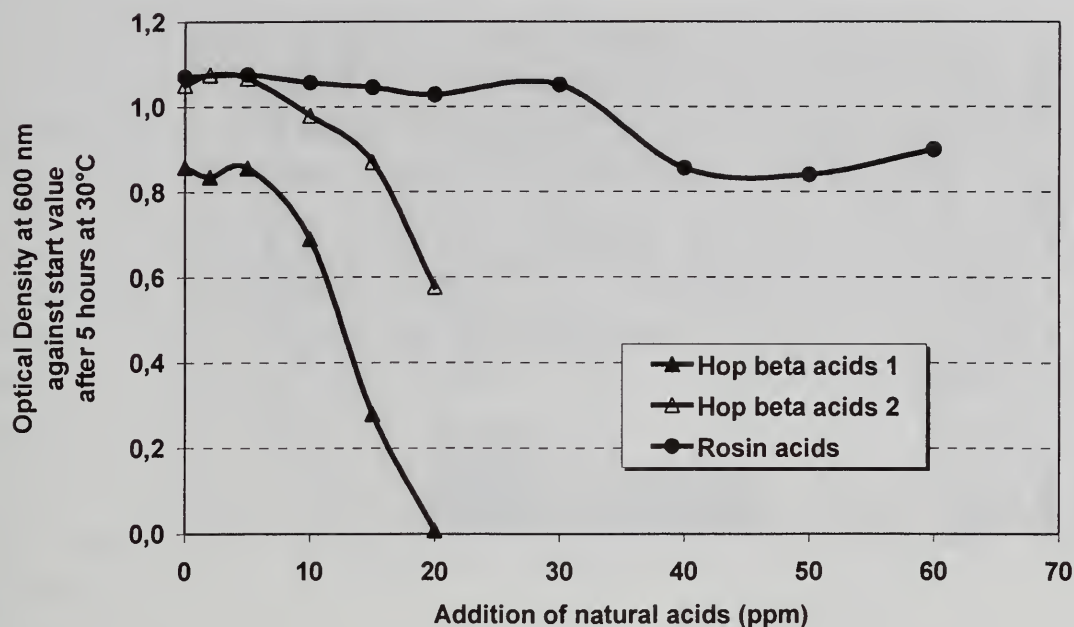


Figure 20. Preliminary aerobic trials with *Leuconostoc* and addition of natural acids.

Results from full scale trials

The first full scale trials with rosin acids were started during the 20002 campaign at Tulln. This factory operates one tower, together with three mixers, and the total slicing capacity is above 11.000 tons per day, if beet pulp can be pressed well. Dry matter of pulp is very important for the slicing capacity and it is often bad during the first part of the campaign. As lactic acid improves pulp pressing, only just "traces" of biocides are allowed if pulp pressing works badly. Nearly no effect is visible during this time, but only 3,5 ppm of hop beta acids had a good effect after 20 days of campaign (Figure 21). In the second part of the campaign, when dry matter of pulp went up to 30 % and more, we were able to lower lactic acid with 12 and later 18.5 ppm of rosin acids to a level of about 400 mg/L during the dosing periods (Figure 21). The last period was used to take samples for residue studies. We did not present the effect of rosin acids without at least one repetition in a following campaign.

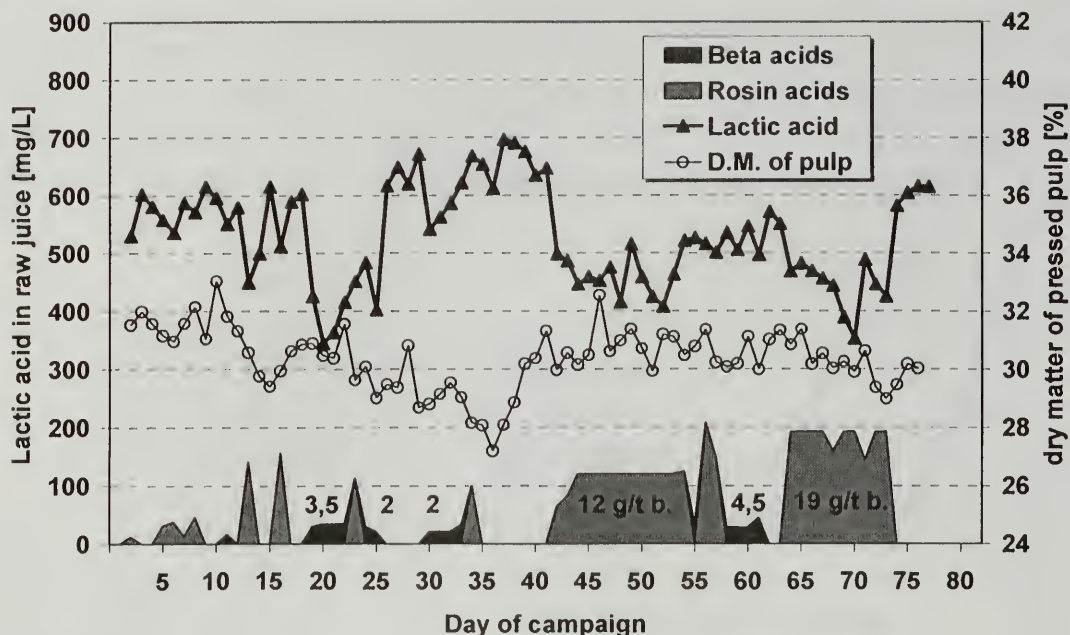


Figure 21. First full scale application of rosin acids in Tulln (2000).

Pulp pressing was bad during the first part of the campaign 2001, and no biocides were allowed, in order to keep the beet slicing capacity. But during the second half of the campaign several alterations with dosing, without dosing and with dosing of BetaStab were possible (Figure 22). Lactic acid dropped to 400 mg/L with 20 ppm of rosin acids, but only short effects were reached with 3 ppm of hop beta acids. This was in agreement with earlier findings for the factory Tulln, as reported at Antwerp [11]. For a short time we used 6 ppm of hop beta acids, which was equal to 20 ppm of rosin acids.

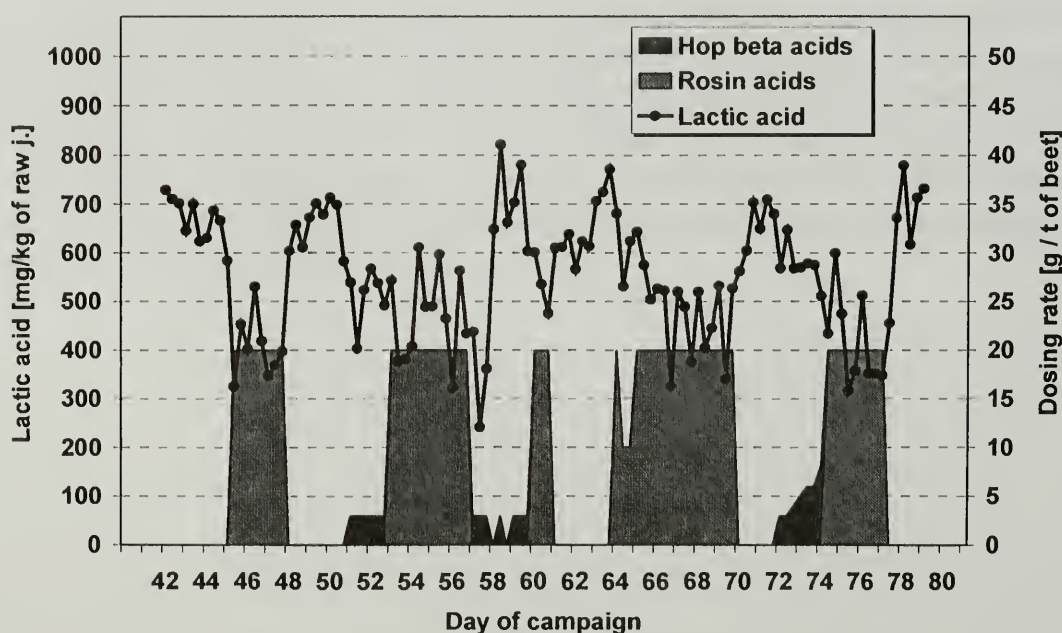


Figure 22. Full scale repetition in Tulln: Lactic acid control (2001).

The dosing periods show a rather diagonal trend in lactic acid levels. This may be caused by killing of biofilms on inner surfaces of the tower and the three mixers in course of time. As trials require alterations between dosing- and blank-periods for reasons of demonstration, we allowed for a recovering of such biofilms during blank periods unintentionally. Thus higher lactic acid levels have to be accepted or higher dosing rates were necessary, compared to conditions without trials.

The sugar factory Leopoldsdorf was surprised to have high levels of NO_2 in raw juice after several years. We started to use rosin acids to the two existing towers at the lowest possible position. Due to a dosing failure the dosing rate was very low for rosin acids and no significant effect was visible (Figure 23). After a blank period the mixer was chosen as new dosing point and dosing was increased to 12,5 ppm - with a large effect on NO_2 and no effect on lactic acid. After a new blank period 3 ppm of hop beta acids were used, with a large effect on both NO_2 and lactic acid. After a further blank period, 15 ppm of rosin acids were used and the nitrite forming organisms were knocked out for the rest of the campaign.

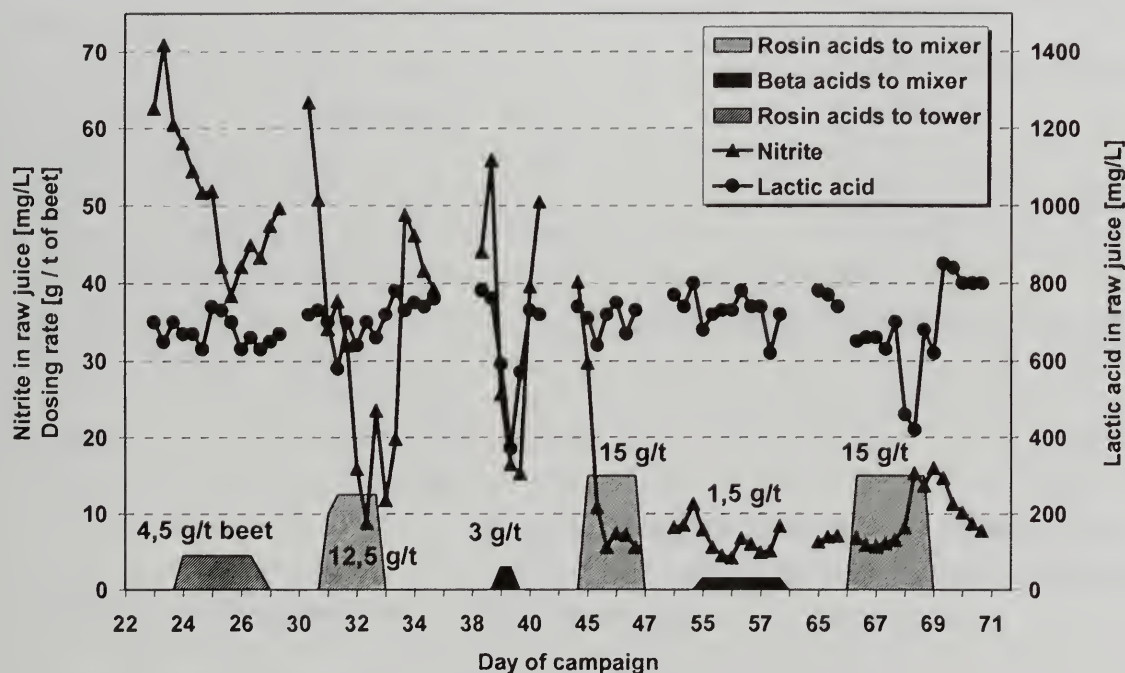


Figure 23. Nitrite and lactic acid control at Leopoldsdorf factory (2001).

The lowering of lactic acid by 3 ppm of hop beta acids had a bad effect on pulp pressing and energy targets of the factory. Therefore the next dosing was cut in half and this low dosing did not show any effect. A last period with 15 ppm of rosin acids resulted in a lowering of lactic acid, but we cannot explain the difference to the earlier trial with the same dosing. But the valuable effect of hop beta acids on NO_2 reduction was confirmed for rosin acids in this factory.

First results on rosin acids residues

Table 2 shows some preliminary figures on residues. The behavior is similar as for hop beta acids. The rosin acids are found partly in the raw juice and the other part is precipitated and going to pulp. Because of the low amount of pressed pulp, compared to raw juice, the concentration is accumulated. A lot of rosin acids are removed during juice purification, the rest is accumulated during evaporation and crystallization and found mainly in molasses. As rosin acids are not effective at low temperatures, no danger for molasses fermentation should occur. If traces in white sugar are compared with retsina wine, the amount of consumption has to be considered. We also have found traces of rosin acids in chewing gum with turpene resin declaration on the package, which was probably produced with rosin acid glycerol ester.

Table 2. Rosin acids residues after dosing of 16 g / t of beet

<i>SAMPLE</i>	<i>MEAN VALUE</i>
Raw Juice	3,9 ppm
Pulp 30% Dry M.	68 ppm
Thin Juice	0,65 ppm
Thick juice	3,8 ppm
Molasses	22 ppm
White Sugar	60 ppb
Retsina	first result: 4,5 ppb
Chewing gum	first result: 238 ppb

CONCLUSION

- New biocides of natural origin have been discussed.
- Hop beta acids have shown particular effects since 1994.
- Rosin acids show great promise to become a second natural biocide for the sugar industry.
- Both natural acids show similar good effects on thermophiles, compared to mesophiles.
- Rosin acids cause higher dosing rates and residues, compared to hop beta acids.
- Lactic acid control seems to be stable with rosin acids, even under severe conditions.
- Leuconostoc inhibition by hop beta acids is a question of further studies.

APPENDIX: DETAILS ON MATERIALS AND METHODS

- Pure strains of *Bacillus stearothermophilus* (now *Geobacillus stearothermophilus*), such as ATCC 12980 = DSM 22, L33-65 = DSM 457, NCA 1503 = DSM 2027 and of *Leuconostoc mesenteroides*, *subsp. mesenteroides* (ATCC8293 = DSM 20343) have been ordered from the German type culture collection (DSMZ) and activated as recommended in the catalogue of strains [35].
- Deep-frozen raw juice samples from the Agrana-factory Tulln have been used as inoculum for trials with impure laboratory cultures (20 mL / 500 mL).
- Medium "VIII" was composed according to *Bartelmus* and *Perschak* [36], but without sugar, having the following composition per Liter: Bacto-peptone 10 g, yeast extract 5 g, meat extract 5 g, $K_2HPO_4 \cdot 3H_2O$ 1,3 g, $MgSO_4 \cdot 7H_2O$ 0,1 g, $FeSO_4 \cdot 7H_2O$ 0,02 g, pH adjusted to 7. Sucrose (2,5 g/500 mL of culture) was added as sterilized aqueous solution (40 %) at the starting time of trials with pure strains.
- MRS medium was used for *Leuconostoc mesenteroides* as recommended in the DSMZ catalogue [35] for a first cultivation to an active culture. pH was adjusted to 6,5 before inoculation. 10 % of sucrose (insterile) was added prior to the aerobic growth phase to enable dextran formation.
- Pure rosin acids for microbiological trials and analytical standards were ordered from Helix-Biotech, New Westminster, Canada.
- Hop beta acids were used as 10 % aqueous solution BetaStab10A.
- Colophony, quality WW from Portugal, was used for trials. The material was ground and dissolved in ethanol or dissolved in water by titration with NaOH to sodium rosinate (10,7 % rosinate, corresponding to 10 % of rosin acids).
- Laboratory trials were carried out in sterile glass vessels (500 mL) with pH recording and slow magnetic stirring, in order to avoid artificial aeration and to simulate conditions in extraction towers. In addition to the most important information from pH-recording, samples were drawn for measurement of optical density and microscopy.
- Preliminary trials with *Leuconostoc* were carried out in a shaker at 30°C in dented Erlenmeyer flasks. The pH of the medium was adjusted to 6,5 before inoculation. After growing on of the inoculum, 10 % sucrose were added prior to the aerobic growth phase (5 hours) to enable growth and dextran formation.
- Optical density was measured against air at 600 nm and reported as difference against substrate.
- Bacterial cells were stained by a vital staining method, the Congo red method [34], but with 1 cm² film area instead of 10 cm², as reported in the original paper.
- Raw juice samples from full scale trials were analyzed for lactic acid by the Yellow springs equipment and for nitrite with the colorimetric method, based on sulfanilic acid and alphanaphthylamine [2].
- Rosin acid residues were determined with an existing DIONEX system: RP-column Nucleosil C-18 (Marcherei-Nagel) with appropriate pre-column. Eluent: acetonitril-water. Gradient elution from 60:40 to 90:10 within 60 min. Flow rate 1 mL/min at 25°C. UV/VIS detection at 208/245 nm. The isomers were not separated from each other and till now the common peak was calculated as abietic acid. Additionally the proportion

between isomers and the separated dehydroabietic acid was used to check the preliminary results.

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BENCH SCALE STUDIES TO EVALUATE THE EFFECTIVENESS OF SEVERAL BIOCIDES AND CHEMICALS WITH COMPARISONS TO FACTORY TRIALS

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ABSTRACT

The evaluation of the effectiveness of biocides in the factory alone is difficult due to factory operations such as other additives, temperature, etc. interfering with results. Therefore, several bench scale studies were carried out with the use of raw juice prepared in the pilot plant at American Crystal Sugar Company's Technical Center without the use of additives. Biocides Kcide-800 (sodium dimethyldithiocarbamate), Tsunami-100, (peroxyacetic acid/hydrogen peroxide) Kcide-850 (50% glutaraldehyde) and chemicals – sodium sulfite, sulfur dioxide, and ammonium bisulfite – were evaluated at different concentrations to determine biocidal efficacy on microbial population. These studies were followed by a trial with use of these biocides at one of the company's factories.

These studies showed that biocides such as Kcide-800 and Kcide-850 are effective when microbial counts are at moderate levels of log 4.5 to 5.0 cfu/g for mesophiles and log 3.0 cfu/g for the thermophiles. However, these biocides were ineffective in decreasing counts during high microbial loading of log 7.0-8.0 for mesophiles.

Tsunami-100 was not as effective as Kcide-800 or Kcide-850. It showed significant biocidal effect around 80 ppm on moderate levels of microbial populations.

Sulfur dioxide was effective or better on moderate levels of microbes at ~200 to 400 ppm concentration. SO₂ had an advantage over other biocides in that it could be used at higher concentration (above 400 ppm) to significantly decrease high microbial loading. Sodium sulfite was found to have no effect on moderate levels of mesophiles at concentration similar to SO₂. Also thermophiles were decreased only by one log unit. Ammonium bisulfite at similar concentrations of SO₂ to gaseous SO₂ had much less biocidal effect on mesophiles and thermophiles at moderate microbial levels.

INTRODUCTION

Several bench scale studies were carried out from March to May 1999 with the use of raw juice prepared at the pilot plant at American Crystal Sugar Company's Technical Services Center without the use of additives. A number of biocides (Kcide-800, Tsunami-100, Kcide-850) and chemicals (sodium sulfite, sulfur dioxide, and ammonium bisulfite) were evaluated at different concentrations to determine biocidal efficacy on microbial populations. These studies were followed by a trial with use of these biocides at the Crookston factory.

MATERIALS & METHODS

A) BIOCIDES – Three different biocides were evaluated.

1. Kcide-800 (Kabo Chemicals) with a specific gravity of 1.176 – The active ingredients being sodium dimethyldithiocarbamate (15-16%), and Nabam or disodium ethylene-bisdithiocarbamate (15-16%). This biocide was evaluated at 20, 40, 100, and 400 ppm (and with corrections for specific gravity it was evaluated at 23.52, 47.04, 117.6, and 470.4 ppm respectively).
2. Tsunami-100 (Ecolab) with a specific gravity of 1.11 – The active ingredients being peroxyacetic acid (15%) and hydrogen peroxide (11%). This biocide was evaluated at 5.0 and 80 ppm concentrations (and with corrections for specific gravity at 5.55 and 88.8 ppm respectively).
3. Kcide-850 (Kabo Chemicals) with a specific gravity of 1.129 – The active ingredient being glutaraldehyde (50%). This biocide was evaluated at 20, 250, and 500 ppm (and with corrections for specific gravity at 22.58 ppm, 282.25 ppm, and 564.5 ppm respectively).

B) CHEMICALS – Three different chemicals were evaluated.

1. Sodium sulfite (Na_2SO_3) – This was evaluated at 167.6 and 327.5 ppm concentration on titration or 200 and 400 ppm concentration on weight basis.
2. Ammonium bisulfite (NH_4HSO_3), 45% solution with a specific gravity of 1.25 – This was evaluated at 200, 400, 600, and 800 ppm concentrations (and with corrections for specific gravity at 249.75, 499.5, 749.25, and 999.0 ppm respectively).
3. Sulfur dioxide (SO_2) – This was sparged into raw juice as close to 200 and 400 ppm as possible and evaluated at 195.5 and 363.8 ppm concentrations.

C) EXPERIMENTS – A number of bench scale biocide studies were carried out with Kcide-800 (5 studies), Tsunami-100 (3 studies), Kcide-850 (3 studies), sodium sulfate – Na_2SO_4 (1 study), Sulfur dioxide – SO_2 (1 study), and ammonium bisulfite – NH_4HSO_3 (2 studies). The initial Kcide-800 and Tsunami-100 studies were preliminary studies to find suitable parameters for further studies. Therefore only the latter studies with similar parameters will be used in main discussions with minor references to other experiments.

1. Raw Juice – The raw juice was prepared at the Technical Services Center pilot plant without the use of any additives on 1/8/99 and used in all evaluations. This raw juice was

poured into one gallon containers and kept frozen in a walk-in freezer at -15°F. Single gallon containers of juice were thawed out for $\approx 1\frac{3}{4}$ days and used as required for experiments. The time of $1\frac{3}{4}$ days for thawing and storing of juice at ambient temperature in the lab ($\approx 25^\circ\text{C}$) was found to give a sufficient microbial level (mesophiles log 4.5 to 5.0 cfu/g and thermophilic log 3.0 cfu/g) for biocide evaluations. In other preliminary studies the raw juice was thawed and stored for shorter and longer periods of time (1, $2\frac{3}{4}$, $3\frac{3}{4}$, $4\frac{3}{4}$ days).

2. Time and Duration for Incubation of Sample Bottles – Sample bottles with different concentrations of biocides and control bottles (without biocide) were incubated at 40°C for 5 hours. In a few preliminary trials test samples were incubated at 25°C for 30 minutes.
3. Microbial Counts – Initial mesophilic and thermophilic counts in raw juice after thawing at the start of experiment (before addition of biocide or incubation of bottles) was obtained. This was followed by obtaining another set of microbial counts (mesophiles, thermophiles) in control bottle (no biocide) and other biocide treated sample bottles after incubation of the bottles for 5 hours at 40°C or 30 minutes at 25°C during initial experiments. The raw juice samples were evaluated for yeast and mold as well, but these organisms were found to be absent in all the raw juice experiments after processing and freezing.

D) BIOCIDE TRIALS AT ACS FACTORY

- This trial was carried out at one of the ACS factories for five days from 4/29 through 5/3/99. Samples for microbial evaluations (mesophiles, thermophiles, Leuconostoc, yeast and mold) were taken from five locations in the factory. These were: diffuser cell 1, diffuser cell 5, raw juice surge tank, raw juice before heater, and the pulp press water tank.
- Further factory trials with addition of Kcide-800 before and after the slicer are in progress.

RESULTS AND DISCUSSION

The controlled experimental conditions of thawing raw juice for $1\frac{3}{4}$ days and incubation of control and biocide treated bottles at 40°C for 5 hours were carried out for the following:

- Kcide-800 (thiocarbamate) – Two experiments carried out on 3/30/99 and 4/7/99, respectively.
- Tsunami-100 (H_2O_2 and per acetic acid) – Two experiments carried out on 3/24/99 and 6/9/99, respectively.
- Kcide-850 (glutaraldehyde) – Two experiments carried out on 5/12/99 and 6/9/99, respectively.

In addition, single experiments with the above controlled conditions were carried out for SO_2 with samples of raw juice on 4/7/99 and sodium sulfite (Na_2SO_3) on 4/7/99. Also two experiments were carried out for ammonium bisulfite on 5/12/99 and 7/28/99. A summary of biocides and chemicals used in bench studies with concentrations evaluated is given in Table 1.

In the case of thiocarbamate (Kcide-800) the mesophilic count showed a decrease of 3 log units and thermophilic counts, a decrease of 2 log units at 23.52 ppm and 47.04 ppm as compared to control when incubated at 40°C for 5 hours. These results were the same in both experiments carried out on 3/30/99 and 4/7/99. See Table 2 for detail.

The Tsunami-100 was evaluated at concentrations of 5.55 ppm and 88.8 ppm. This biocide was found to have no effect on mesophilic and thermophilic populations at ≈ 5 ppm concentration in both experiments of 3/24/99 and 6/9/99. However, the 88 ppm concentration showed greater than 1 log unit decrease in mesophilic counts and 1.5 log unit decrease in thermophilic counts as compared to control in experiments of 3/24/99. However, in the second experiment (6/9/99) the 88 ppm concentration of Tsunami-100 had no effect on mesophiles and showed 0.5 log unit decrease in thermophilic count. See Table 3 for detail.

In the case of Kcide-850 (glutaraldehyde), this showed a decrease of mesophilic counts of greater than 3 log units and decrease of thermophilic counts of 1.5 log units at 22.58, 282.25, and 564.5 ppm concentration in experiment on 5/12/99. In the second experiment on 6/9/99 only a concentration of 22.58 ppm which is close to the maximum concentration permitted for use (20 ppm) was evaluated. Here mesophilic counts decreased by greater than 4 log units and thermophilic counts decreased by one log unit at a concentration of 22.58 ppm. See Table 4 for detail.

However, interestingly in preliminary Kcide-800 experiments (3/15/99, 3/16/99, 3/22/99) and preliminary Tsunami-100 experiment (3/17/99) when raw juice was allowed to thaw and be kept at ambient temperatures for $2\frac{3}{4}$ to $4\frac{3}{4}$ days giving initial mesophilic counts in log 7.8 to 8.5 range and thermophilic counts in the log 3 cfu/g range. (See Table 2) No significant decrease in mesophilic or thermophilic counts at 23.52, 47.04, 117.6, and 470.4 ppm of Kcide-800 or 5.5 and 88.8 ppm concentration of Tsunami-100 was observed. This is probably due to the fact that the microbial loading of mesophiles (log 7.8 to 8.5 cfu/g) and thermophiles (log 3 cfu/g) are too high for the biocide to have any impact on decreasing numbers of microbes. It is also probably due to the fact that the microbes have reached the stationary phase of growth in the life cycle where the number of cells produced is equal to the numbers dying off giving a flat curve. This phase of growth is also probably less susceptible to biocidal activity unlike the growth phase where there is active multiplication of cells with increase in numbers.

In the bench scale studies with chemicals containing SO₂ the following was observed. See Table 1 for detail. For instance in the sparged SO₂ experiment of 4/7/99 the mesophilic counts decreased by >2.5 log units and thermophilic counts by >1 log unit at 195.5 ppm concentration as compared to the control. Also at a higher concentration of 363.8 ppm SO₂ the mesophilic counts decreased by 3 log units and thermophilic counts decreased by >1 log unit (which was similar to the result at 195.5 ppm concentration).

Na₂SO₃ was less effective than sparged SO₂. See Table 5 for detail. For instance in the experiment carried out on 4/7/99 there was no significant decrease in mesophilic counts at 167.6 ppm or 327.5 ppm concentration. However, thermophiles showed a decrease of about 1 log unit at 167.6 ppm and 327.5 ppm concentration.

In the case of ammonium bisulfite (NH_4HSO_3) on experiments carried out on 5/12 and 7/28/99 (See Table 6) the mesophiles decreased by greater than 1 to 1.5 log units and thermophiles by 1 to greater than 1.5 log units at 249.75 ppm NH_4HSO_3 concentration or 161.34 ppm SO_2 concentration. At 499.5 ppm NH_4HSO_3 or 322.67 ppm SO_2 concentration slight increases in kill levels of mesophiles (greater 1.5 to ≈ 2 log units) and thermophiles (1.5 to >1.5 log units) was observed on 5/12 and 7/28/99 bench scale studies. In addition on 7/28/99 higher concentrations of NH_4HSO_3 of 749.25 ppm (or 484.02 ppm SO_2) and 999.0 ppm (or 645.36 ppm SO_2) was evaluated. However, these higher levels of NH_4HSO_3 concentrations gave similar kill rates of ≈ 2 log units for mesophiles and greater than 1.5 log units for thermophiles which was very similar to the kill at 499.5 ppm NH_4HSO_3 (or 322.67 ppm SO_2). This showed that gaseous SO_2 at 363.8 ppm concentration had greater biocidal effect on mesophiles (3 log units) than NH_4HSO_3 at 999 ppm concentration or as 645 ppm SO_2 .

BIOCIDE FACTORY TRIAL

A biocide trial to evaluate the effectiveness of Kcide-800 (thiocarbamate) and Kcide-850 (glutaraldehyde) without the use of SO_2 was begun at Crookston factory on 4/29/99 and continued through 5/3/99. Samples were taken for microbial assessment (mesophiles, thermophiles, *Leuconostoc*, yeast and mold) daily through the trial period. Five locations in the factory were evaluated. See Figures 1-5 for changes in microbial loading at press pulp water tank, raw juice before heater, raw juice surge tank, diffuser cell 5, and cell 1 during the trial period.

In this study SO_2 was continuously added to cell 5 of the diffuser (Silver DDS Slope diffuser) through the trial period. However, SO_2 was shut off in pulp press water (PPW) after the initial samples were taken and Kcide-800 was added instead. This resulted in drastic increases in mesophilic counts of greater than 2 log units (log 5.2 to log 7.56 cfu/g) in the pulp press water tank. A very significant increase in thermophilic counts of greater than 2.5 log units (from log 3.31 to log 5.89 cfu/g) in PPW tank was also observed. These changes were seen within 24 hours of addition of Kcide-800 to PPW and also resulted in a significant increase in L-lactic acid numbers from 299 to 1295 ppm at this location. However, *Leuconostoc* and yeast and mold counts did not show significant changes in numbers with the addition of Kcide-800. Also, due to the rapid increase in lactic acid on 4/30/99 both the addition of Kcide-850 (glutaraldehyde) and SO_2 was begun at the PPW location simultaneously though advised not to do so previously. Chemicals such as SO_2 and glutaraldehyde should never be added simultaneously due to the formation of the bisulfite addition product resulting in the unavailability of these biocides to act on microbes and have any biocidal effect. SO_2 was added to PPW continuously from 5/1/99 through 5/3/99. However, it took a couple of days before the mesophilic and thermophilic counts went back to the levels they were on 4/29/99 at the start of the experiment. The effect of the addition of Kcide-800 to PPW through a 24-hr period was seen in other regions with significant increases in thermophilic counts of greater than one log unit in locations such as the raw juice before heater, raw juice surge tank, and diffuser cell 5 on 4/30/99. This also resulted in corresponding significant increases in lactic acid values from ≈ 200 ppm to an increased range of approximately 972-1430 ppm in these regions. However, presently Kcide-800 is being applied directly on beets before and after the slicers and has shown promise. Further testing is in progress.

CONCLUSIONS

These studies have shown that:

- 1) Biocides such as Kcide-800 (thiocarbamate) and Kcide-850 (glutaraldehyde) are effective when microbial counts are at moderate levels of log 4.5 to 5.0 cfu/g for mesophiles and log 3.0 cfu/g for thermophiles. However, these biocides are ineffective or incapable of decreasing counts during high microbial loading such as log 7.0-8.0 for mesophiles (see Table 2). This is probably due to the maximum concentration of biocide permitted for use in the factory for Kcide-800 is 20 ppm and for Kcide-850 is 20-500 ppm. This is also probably one of the reasons that the Crookston factory got out of control within 24 hrs of use of Kcide-800 during trials carried out from 4/29 to 5/3/99. Another reason being that Kcide-850 and SO₂ was added simultaneously producing the bisulfite addition product and therefore the biocides were unavailable for control or decrease of microbes.
 - Further trials with addition of Kcide-800 directly on beets before and after the slicer are being carried out and have shown promise. Further testing is being carried out.
- 2) Tsunami-100 was found not to be as effective as Kcide-800 or Kcide-850. Also it showed significant biocidal effect only around 80 ppm on moderate levels of microbial populations.
- 3) Sulfur dioxide was found to be as effective or better on moderate levels of microbes at ≈200 to 400 ppm concentration. Also, SO₂ has an advantage over other biocides in that it may be used at higher concentrations (above 400 ppm) to significantly decrease high microbial loading.
- 4) Sodium sulfite (Na₂SO₃) was found to have no effect on moderate levels of mesophiles at concentrations similar to SO₂ (≈200 to 400 ppm). Also thermophiles were decreased only by about one log unit.
- 5) Ammonium bisulfite (ABS) at similar concentrations of SO₂ to gaseous SO₂ had much less biocidal effect on mesophiles and thermophiles at moderate microbial levels (see Table 1). This is similar to findings at British Sugar (Verbal communication – they did not have much luck with ABS). Also the cost of ammonium bisulfite is 3-5 times higher than gaseous SO₂.

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TABLE 1
BIOCIDES AND CHEMICALS USED IN BENCH STUDIES

Biocide / Chemical	# of Studies	Concentrations Evaluated (ppm)
Dithiocarbamate (Kcide – 800) Kabo Chemicals	5	20, 40, 100, 400 23.52, 47.04, 117.6, 470.4
Peroxy Acetic Acid / Hydrogen Peroxide (Tsunami-100) Eco Lab	3	5, 80 5.55, 88.8
Glutaraldehyde (50%) (Kcide-850) Kabo Chemicals	3	20, 250, 500 22.58, 282.25, 564.5
Sodium Sulfite (Na_2SO_3)	1	200, 400 167.6, 327.5
Ammonium Bisulfite (NH_4HSO_3) – 45% Solution	2	200, 400, 600, 800 249.75, 499.5, 749.25, 999.8
Sulfur Dioxide (SO_2)	1	200, 400 195.5, 363.8

TABLE 2
BIOCIDE BENCH STUDIES
MICROBIOLOGICAL RESULTS WITH KCIDE-800

Biocide	Trial Type & Date	Types of Microbes	Raw Juice Initial Log Count (cfu/g)	Control Log Count (cfu/g)	Microbial Count in Different Concentrations of Biocide Used (ppm)		
					23.52	47.04	117.6
Kcide 800 Dithiocarbamate	Trial 1 (1 ¾ Days) 3-30-99	Mesophiles	4.58	6.68	3.70 (avg)	3.70 (avg)	-
		Thermophiles	1.78	3.48	1.24 (avg)	1.24 (avg)	-
Kcide 800	Trial 2 (1 ¾ Days) 4-7-99	Mesophiles	4.83	6.92	3.89 (avg)	-	-
		Thermophiles	3.07	3.33	2.02 (avg)	-	-
Kcide 800	Trial 3* (2 ¾ Days) 3-15-99	Mesophiles	8.03		7.98	7.86	-
		Thermophiles	3.34		3.04	3.15	-
Kcide 800	Trial 4* (3 ¾ Days) 3-16-99	Mesophiles	8.53		-	-	8.46
		Thermophiles	2.95		-	-	3.23
Kcide 800	Trial 5 (2 ¾ Days) 3-22-99	Mesophiles	7.82	8.58	8.24 (avg)	8.26 (avg)	-
		Thermophiles	3.51	2.18	2.15 (avg)	2.15 (avg)	-

* In these 2 trials (#3 & #4) the samples were incubated for 30 minutes at 25°C after addition of the biocide Kcide-800. (In all other trials - #'s 1, 2, & 5, the samples were incubated for 5 hrs at 40°C.)

NOTE: The days in parentheses in Column 2 refer to the time the frozen raw juice was thawed out for use in trials.

TABLE 3
BIOCIDE BENCH STUDIES
MICROBIOLOGICAL RESULTS WITH TSUNAMI-100

Biocide	Trial Type & Date	Types of Microbes	Raw Juice Initial Log Count (cfu/g)	Control Log Count (cfu/g)	Microbial Count in Different Concentrations of Biocide Used (ppm)	
					5.55	88.8
Tsunami-100 Peroxy acetic acid & hydrogen peroxide	Trial 1 (1 ¾ days) 3-24-99	Mesophiles	4.97	7.37	7.38	6.24
		Thermophiles	3.86	3.87	3.77	2.30
Tsunami-100	Trial 2 (1 ¾ days) 6-9-99	Mesophiles	4.94	8.32	8.40 (avg)	8.26 (avg)
		Thermophiles	2.20	3.18	3.36 (avg)	2.66 (avg)
Tsunami-100	Trial 3* (4 ¾ days) 3-17-99	Mesophiles	8.46	-	8.58	8.49
		Thermophiles	3.51	-	3.08	2.30

* In Trial #3 with Tsunami-100 the sample was incubated for 30 min. at 25°C (while in all other trials the samples were incubated for 5 hrs at 40°C).

NOTE: The days in parentheses in Column 2 refer to the time the frozen raw juice was thawed out for use in trials.

TABLE 4
BIOCIDE BENCH STUDIES
MICROBIOLOGICAL RESULTS WITH KCIDE-850

Biocide	Trial Type & Date	Types of Microbes	Raw Juice Initial Log Count (cfu/g)	Control Log Count (cfu/g)	Microbial Count in Different Concentrations of Biocide Used (ppm)		
					22.58	282.25	564.5
Kcide-850 Glutaraldehyde	Trial 1 (1 ¾ days) 5-12-99	Mesophiles	5.21	7.78	3.99 (avg)	3.96	3.97
		Thermophiles	1.60	3.52	2.04 (avg)	1.78	1.90
Kcide-850	Trial 2 (1 ¾ days) 6-9-99	Mesophiles	4.94	8.32	3.94 (avg)	-	-
		Thermophiles	2.20	3.18	2.08 (avg)	-	-
Kcide-850	Trial 3 (1 day) 4-28-99	Mesophiles	4.21	4.37	4.00 (avg)	3.99	4.00
		Thermophiles	2.18	2.04	1.95 (avg)	2.04	1.95

NOTE:

1. The days in parentheses in Column 2 refer to the time the frozen raw juice was thawed out for use in trials.
2. In all above Kcide-850 trials the samples were incubated for 5 hrs at 40°C.

TABLE 5
BIOCIDE BENCH STUDIES
MICROBIOLOGICAL RESULTS WITH SULFUR DIOXIDE AND SODIUM SULFITE

Chemical Tested	Trial Type & Date	Types of Microbes	Raw Juice Initial Log Count (cfu/g)	Control Log Count (cfu/g)	Microbial Counts in Different Concentrations of Chemicals Used (ppm)	
Sulfur Dioxide (SO ₂) Sparged	Trial 1 (1 ¾ days) 4-7-99	Mesophiles	4.83	6.92	195.5	363.8
		Thermophiles	3.07	3.33	4.15	3.92
					2.15	2.08

Chemical Tested	Trial Type & Date	Types of Microbes	Raw Juice Initial Log Count (cfu/g)	Control Log Count (cfu/g)	Microbial Counts in Different Concentrations of Chemicals Used (ppm)	
Sodium Sulfite (Na ₂ SO ₃)	Trial 1 (1 ¾ days) 4-7-99	Mesophiles	4.83	6.92	167.6	327.5
		Thermophiles	3.07	3.33	6.43	6.70
					2.23	2.28

NOTE:

1. The days in parentheses in Column 2 refer to the time the frozen raw juice was thawed out for use in trials.
2. In above trials, the samples were incubated for 5 hrs at 40°C.

TABLE 6
BIOCIDE BENCH STUDIES
MICROBIOLOGICAL RESULTS WITH AMMONIUM BISULFITE

Chemicals Tested	Trial Type & Date	Types of Microbes	Raw Juice Initial Log Count (cfu/g)	Control Log Count (cfu/g)	Microbial Count in Different Concentrations of Chemicals Used (ppm)			
					249.75 (161.34 SO ₂)	499.5 (322.677 SO ₂)	749.25 (484.02 SO ₂)	999.00 (645.36 SO ₂)
Ammonium Bisulfite (NH ₄ HSO ₃)	Trial 1 (1 ¾ Days) 5-12-99	Mesophiles	5.21	7.78	6.56 (avg)	6.0 (avg)	-	-
		Thermophiles	1.60	3.52	2.11 (avg)	2.08 (avg)	-	-
Ammonium Bisulfite (NH ₄ HSO ₃)	Trial 2 (1 ¾ Days) 7-28-99	Mesophiles	4.94	6.91	5.41 (avg)	4.96 (avg)	4.93 (avg)	4.98 (avg)
		Thermophiles	1.70	3.68	1.91 (avg)	1.90 (avg)	1.84 (avg)	1.98 (avg)

NOTE:

1. The days in parentheses in Column 2 refer to the time the frozen raw juice was thawed out for use in trials.
2. In all above NH₄HSO₃ trials, the samples were incubated for 5 hrs at 40°C.

Fig. 1
MICROBIAL COUNTS IN PRESS PULP WATER TANK DURING BIOCIDES TRIAL AT ACS
FACTORY

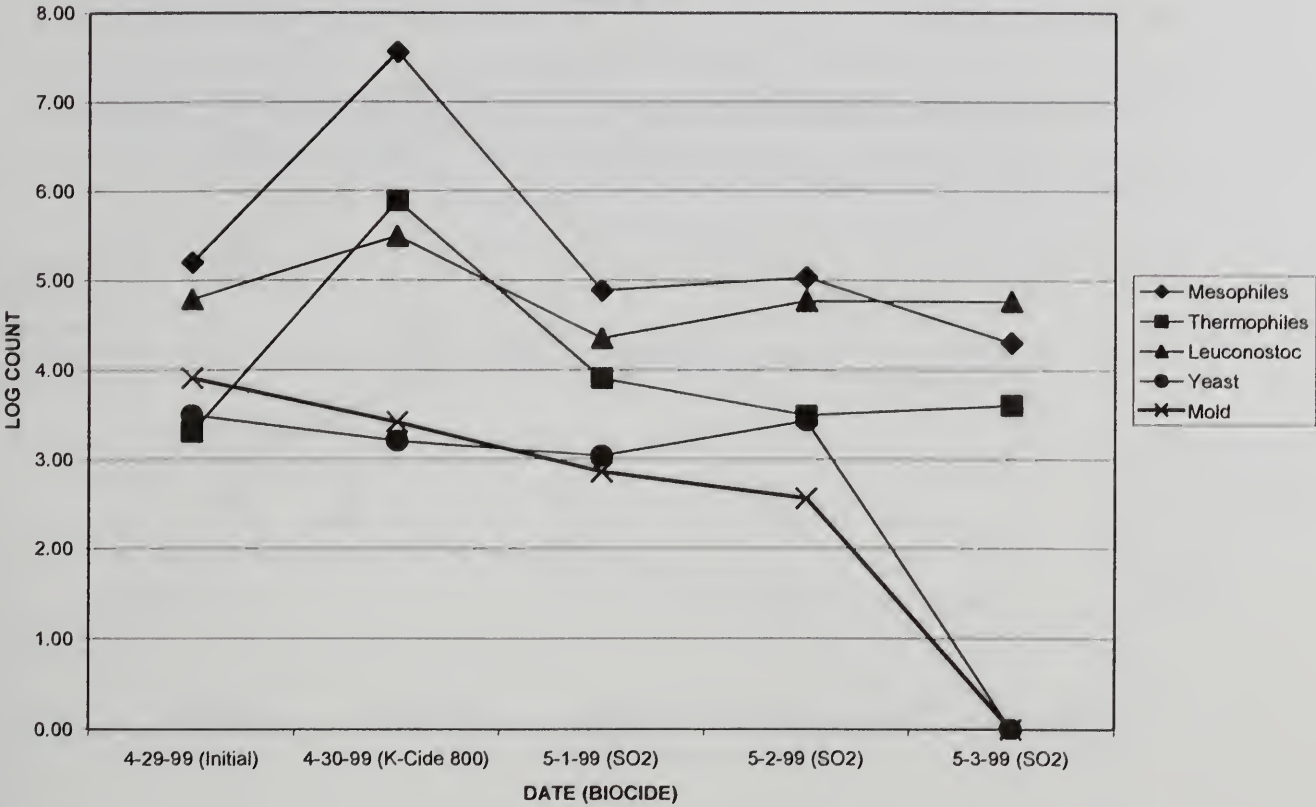


Fig. 2
MICROBIAL COUNTS IN RAW JUICE BEFORE HEATER DURING BIOCIDES TRIAL AT ACS
FACTORY

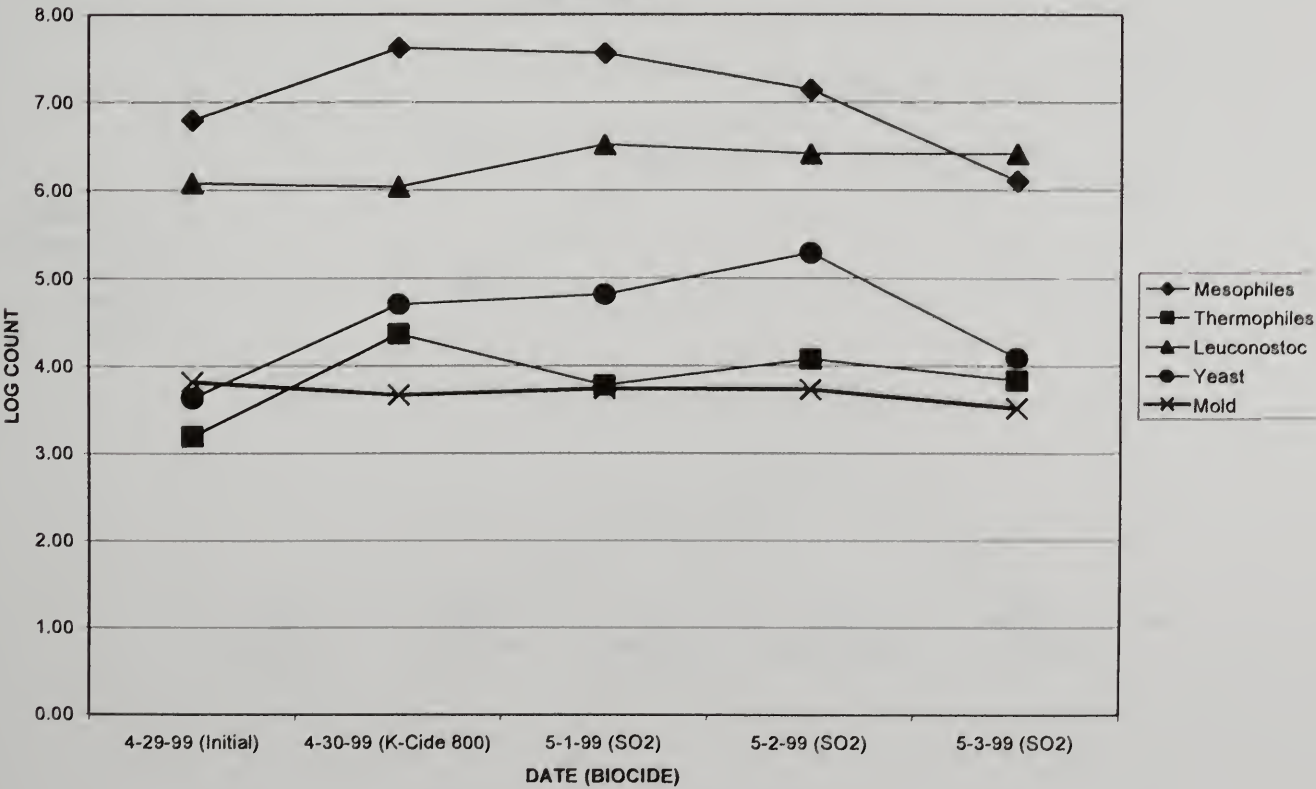


Fig. 3
MICROBIAL COUNTS IN RAW JUICE SURGE TANK DURING BIOCIDES TRIAL AT ACS FACTORY

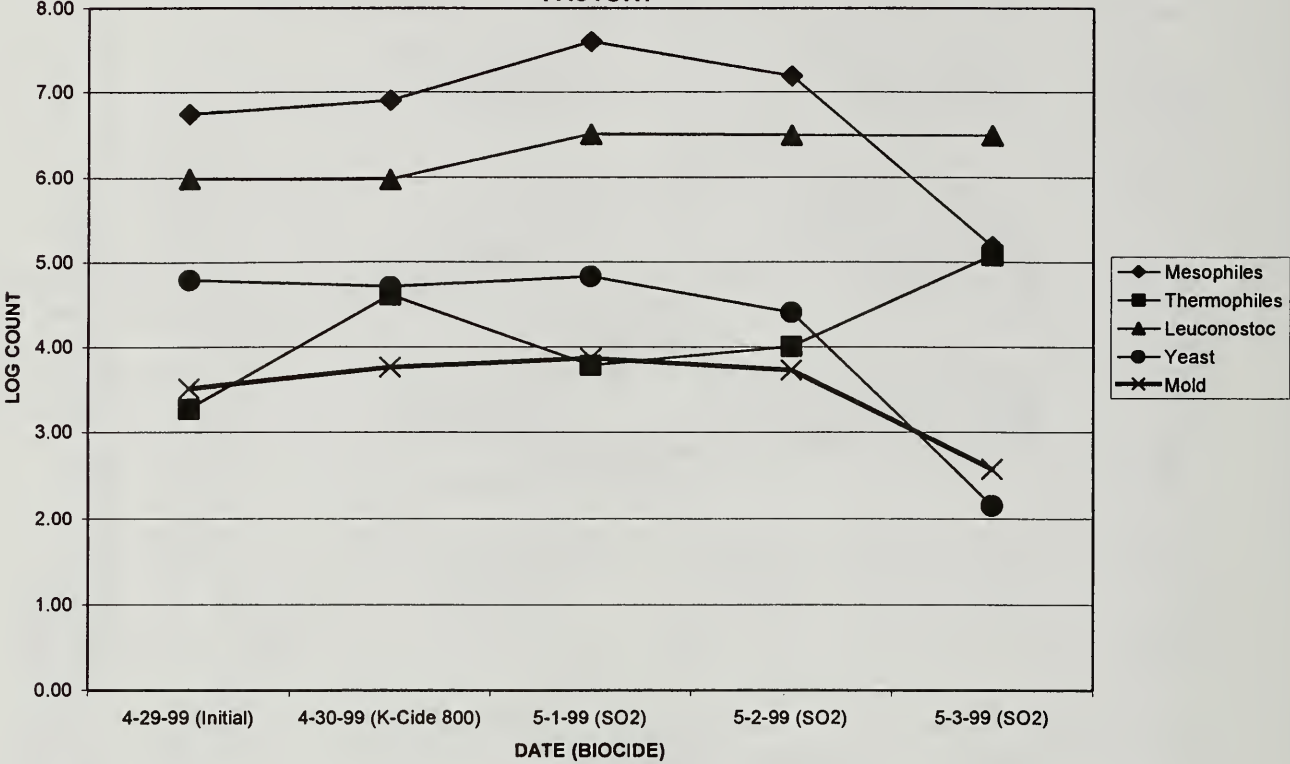


Fig. 4
MICROBIAL COUNTS IN DIFFUSER CELL 5 DURING BIOCIDES TRIAL AT ACS FACTORY

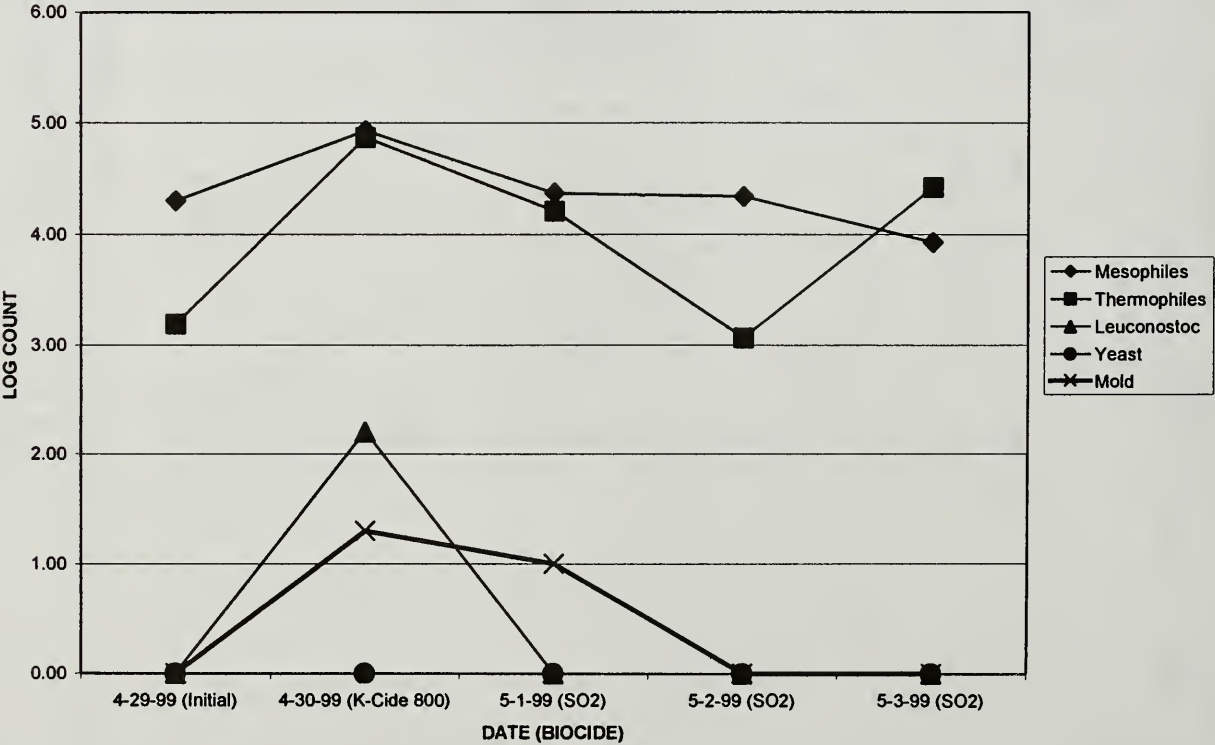
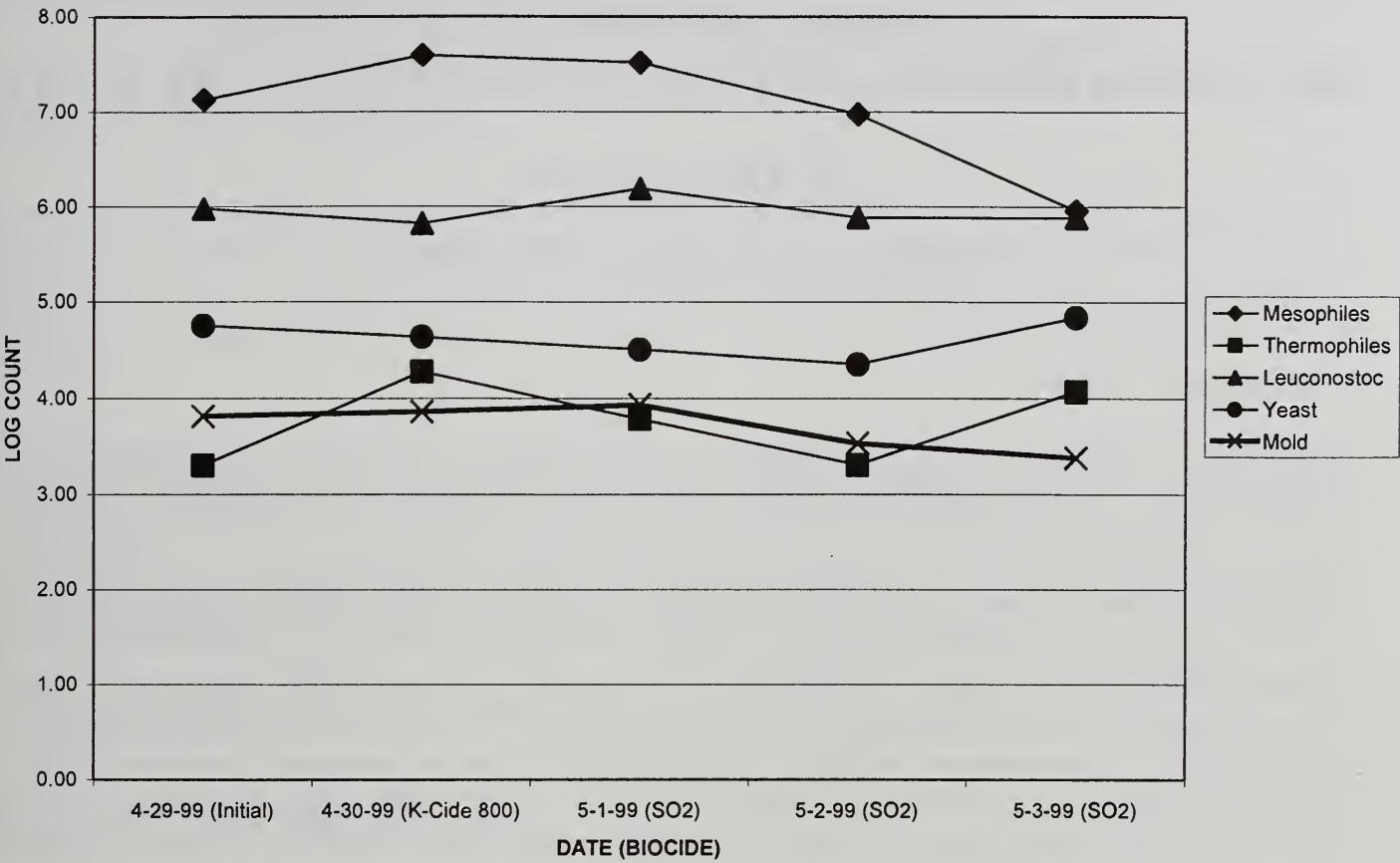


Fig. 5
MICROBIAL COUNTS IN DIFFUSER CELL 1 DURING BIOCIDES TRIAL AT ACS FACTORY



RELATIONSHIP BETWEEN BEET FLUME MICROBIOLOGICAL ACTIVITY AND PROCESS LIME SALTS

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INTRODUCTION

During the 2000-2001 beet processing season factory personnel at one of Amalgamated Sugar's factories observed that a general deterioration of factory operating conditions seemed to correlate with the condition of water in the beet handling (beet flume) and washing system. Deteriorating process conditions included increases in lime salts and related increases in the quantity of soda ash necessary to maintain alkalinity during second carbonation and for realkalization after weak cation exchange thin juice softening. Decreases in thin juice purity and, ultimately, decreases in sugar recovery were also observed, presumably due to increases in the level of non-removable non-sugars, as sodium salts. These changes seemed to coincide with the general increase in dissolved organic components in flume water. Since Amalgamated's factories operate with closed loop flume systems there is no opportunity for removal of dissolved organics other than by oxidation or fermentation and the organic load, therefore, begins to increase dramatically after the first few weeks of factory operation. Because of the coincidental appearance of factory process problems with the increased organic load in flume water it was suspected that conditions in the beet flume were, in fact, influencing operation of the main process stream.

An influence of the beet flume system on process stream chemistry could occur by two possible mechanisms: (1) physical mixing of flume water non-sugars into the process stream or (2) inoculation of beets by the bacterial load in flume water, producing microbiological activity within factory process streams. Other workers have reported that in normal factory operation, depending on beet wash water pH, from 0.4 to 3% of juice non-removable anions may be contributed by physical carry-over of wash water with beets.¹ That fraction of non-sugars would not be expected to cause effects of the magnitude of those observed here but in this case there is the possibility that the factory's aggressive beet cleaning system might lead to surface damage and increased retention of flume water since there is no opportunity for rinsing of beets with clean water. It was feared that excessive flume water carry over, or even an unknown cross-connection, was contributing to excessive levels of non-removable non-sugars in the juice stream. Alternatively, the presence of high bacterial levels in beet, due to inoculation with high-bacteria flume water, might cause generation of non-removable non-sugars from sucrose or invert directly in the juice stream.

To investigate the possible connection of process problems with flume water quality, chemical analysis of various samples from the factory was carried out in the 2000-01 campaign and an extensive program of sample collection for chemical and microbiological determinations was planned for the 2001-02 campaign. Results of the 2000-01 samples, along with preliminary results of the more detailed recent study will be discussed.

ANALYTICAL METHODS

Juice and other liquid samples were collected and, after plating for thermophilic plate count determination, were frozen for later chemical analysis. Cossette samples were washed with buffered distilled water for thermophilic plate count determination, then chopped and frozen for later analysis. Whole beet samples were collected before exposure to flume water, chopped, and frozen for later analysis.

Gas chromatographic (GC) sucrose determinations were made on a HP-5 column (10m X 0.53 mm) at 235°C using salicin [2-(hydroxymethyl)phenyl- β -D-glucopyranoside] as the internal standard.

Organic acids were determined by ion chromatography on a Bio-Rad HPX-87H column using 0.005 N sulfuric acid as the eluent and unsuppressed conductivity detection.

Cation levels were determined by flame atomic absorption spectrometry.

RESULTS AND DISCUSSION

The initial analytical data examined in connection with this study were levels of organic acids, particularly those that generally are formed by microbiological action on sugars and that are not removed during liming and carbonation. These tests were carried out specifically to look for the suspected carry over of beet flume water into the factory juice stream. During the first of such tests, in March of 2001, samples of diffusion juice and thin juice were analyzed for organic acids and results are given in the Table 1. Note the expected increase in lactate from diffusion juice to thin juice, due to the alkaline destruction of invert during purification. Also note that both diffusion juice and thin juice were found to contain detectible levels of butyrate. Since butyrate occurs most frequently under anaerobic fermentation conditions, such as those in ponded flume water, and was not thought to be formed in the aerobic conditions of the juice stream this was taken as preliminary evidence of the carry over of flume water into diffusion juice.

Table 1. Levels of Organic Anions in Diffusion Juice and Thin Juice

Sample	Lactate (meq/l)	Formate (meq/l)	Acetate (meq/l)	Butyrate (meq/l)
Diffusion Juice	2.3	0	13	2.8
Thin Juice	20	3.3	13	1.5

A more extensive series of tests, later in March, 2001, included the analysis of flume water as well as juice stream samples. Results are given in Table 2. Note that the factory under study operates two diffusers (designated A and B) and separate diffusion juice streams are maintained to the prelimer. This data shows organic acid levels for both diffusion juice streams at two points, as well as for thin juice and flume water. First of all, note the very high organic anion load in flume water. The total of measured organic anions is 210 meq/l (or, including calcium as the major cation, roughly 2.0% by weight) and is approximately twenty times the typical level of the same organic anions in the factory juice stream. It is evident that any significant carry over of closed loop flume water into the factory could easily have a significant effect on juice organic anion levels. In this case, butyrate was detected in both diffusion juice streams at the cossette mixer but could not be detected later in diffusion juice or in thin juice. It should be pointed out that, because of long ion chromatographic peak retention time and peak broadening, the butyrate levels reported here are near the limit of detection in juice samples. This set of samples provided only sporadic evidence of flume water carry over into juice but further evidence was provided several months later in connection with an entirely different project.

Table 2. Organic Anions in Flume Water and Factory Process Streams

Sample	Lactate (meq/l)	Formate (meq/l)	Acetate (meq/l)	Butyrate (meq/l)	Total Organic Acids (meq/l)
Flume Water	37	2.0	100	69	210
Diff. Juice A at mixer	2.7	0	6.7	1.7	11
Diff Juice B at mixer	1.8	0	7.3	1.1	10
Diff Juice A at prelimer	2.7	0	5.1	0	7.8
Diff. Juice B at prelimer	1.8	0	6.7	0	8.5
Thin Juice	13	2.6	6.0	0	22

Analysis of a problem-causing condensate from the raffinate evaporators of a chromatographic

Analysis of a problem-causing condensate from the raffinate evaporators of a chromatographic sucrose recovery system revealed the condensate to contain from 140-190 ppm acetic acid and 120-140 ppm butyric acid. This system had been processing molasses from the factory with flume-related problems and it seems likely that butyrate from molasses had evaporated and accumulated in a loop consisting of raffinate condensate going back to the molasses separator as elution water.

Because of the preliminary evidence for carry over of flume water into the factory juice stream, a more thorough study of the entire system was planned for the 2001-02 campaign. Planned sample collection involved sampling at least once monthly (to include early periods of operation with low flume water organics as well as periods with high organic load). A complete set of grab samples from throughout the beet end was planned to include:

- Unwashed beets
- Beets subjected to flume water (cosettes)
- Flume water (from three locations)
- Press water
- Diffuser supply water
- Raw juice (at cosette mixer and prelimer)
- Milk of lime
- Prelimer overflow
- Cold limer
- Hot limer
- 1st Carbonation
- Clarifier overflow
- 2nd Carbonation
- Unsoftened thin juice
- Thin juice
- Thick juice

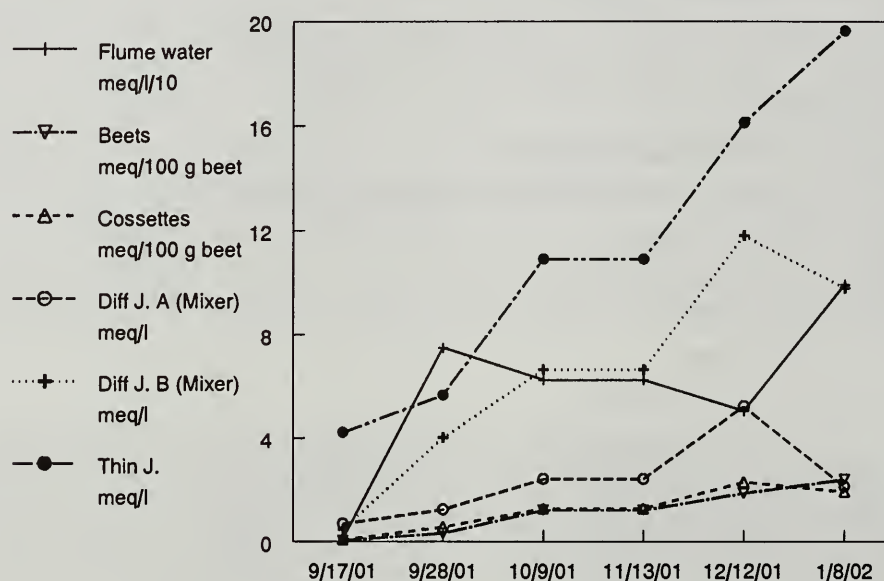
and analytical data was to be collected on all samples for:

- Bacteria levels (where applicable)
- RDS
- Sucrose (by pol and gas chromatography)
- Cations
- Invert
- Raffinose
- Organic anions
- Inorganic anions
- Minimum lime salts (carbonation samples only)

In general, microbiological analysis was carried out immediately after sample collection and samples were then quickly frozen for later chemical analysis.

Evaluation of this data has, so far, been limited to looking, over the length of campaign, for trends in factory juice stream analysis that parallel trends in flume chemistry or microbiological activity. First of all, to examine the question of whether or not flume water carries over into the juice stream, flume water organic anion level trends were compared to comparable levels in factory streams. The first graph, Figure 1, shows levels for organic anions in flume water as well as clean beets, cossettes, raw juice, and thin juice over the six sets of samples collected.

Figure 1
Total Organic Anions



Note that, while organic anions in cossettes trend generally upward to a maximum value on 12/12/01 and anions in thin juice trends upward through the whole campaign, organic acid anions in flume water rise rapidly between 9/17/01 and 9/28/01 as organic loading in the flume went through its usual early-campaign behavior. However, after that point, total acids decrease to a low on 12/12/01 and then rise again. There is nothing in these trends to indicate that factory juice organic anions correlate at all with flume organic anions and, therefore, no evidence of flume water carry over into the juice stream. Levels of total organic anions in cossettes are slightly higher than those in unwashed beet for most sample sets collected (mostly due to slightly higher lactate levels) but further evidence against carry over of organic anions is given by individual anion levels for the day with the highest level of flume anions observed in the study, given in Table 3.

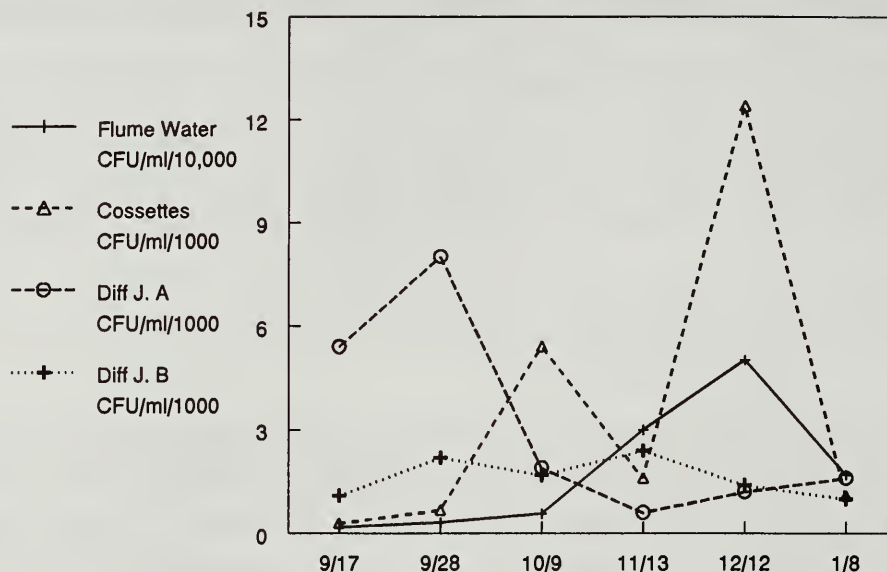
Table 3. Organic Acid Anion Levels on January 8, 2002

Sample	Lactate (meq/l)	Formate (meq/l)	Acetate (meq/l)	Propionate (meq/l)	Butyrate (meq/l)	Total (meq/l)
Flume	9.3	1.8	25	35	33	99
Diff. Juice A (Mixer)	2.1	0	0	0	0	2.1
Diff. Juice B (Mixer)	3.9	0.4	5.5	0	0	9.8
Thin Juice	12	1.9	6.2	0	0	20

The data indicate that, although significant levels of butyrate and propionate were present in flume water, neither anion was detectible in diffusion juice or thin juice. The only anions observed in the juice stream are those present in beets, formed by common juice stream aerobic microbiological activity, or produced by chemical invert destruction. Actually, in contrast to the 2000-01 campaign, none of the samples collected during the 2001-02 campaign shows any detectible butyrate in diffusion juice or thin juice. It should be noted that the worst flume water sample collected (data in Table 3) during the recent study contained only approximately 50% of the levels of butyrate and total organic acids observed in the one grab sample collected during 2000-01 (Table 2 data). Thus, since butyrate was on the borderline of detectible levels in diffusion juice or thin juice that year, it probably would not have been detectible in the recent study unless levels in the flume were, at some point, much higher than any of the samples collected for analysis. Actually, because of the evidence for problems related to flume water quality in the previous year, flume system pH was maintained at a slightly higher level this year. This together with other variables, such as beet quality, seems to have resulted in generally better conditions with respect to organic loading in the flume. Although no unequivocal evidence for carry over of flume water into the process was observed this year, the possibility cannot be ruled out because of evidence obtained last year under poorer flume water conditions.

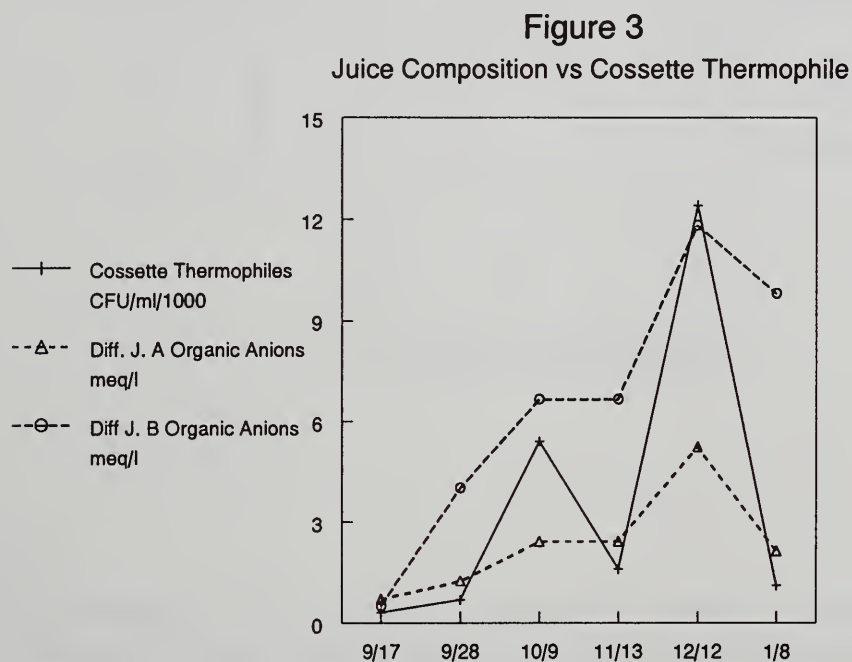
Although no evidence for the carry over of flume water into the factory juice stream was obtained in this year's study, the other possible effect of flume water quality on the process, inoculation of beets with flume water bacteria, was also explored. A further examination of the data was undertaken to look for factory trends related to flume water bacterial levels, especially in the case of thermophilic bacteria which would be most likely to have significant effects on process stream chemical composition. The following graph (Figure 2) shows levels of thermophilic bacteria observed in flume water, cosettes, and diffusion juice over the course of the study.

Figure 2
Thermophilic Bacteria Levels



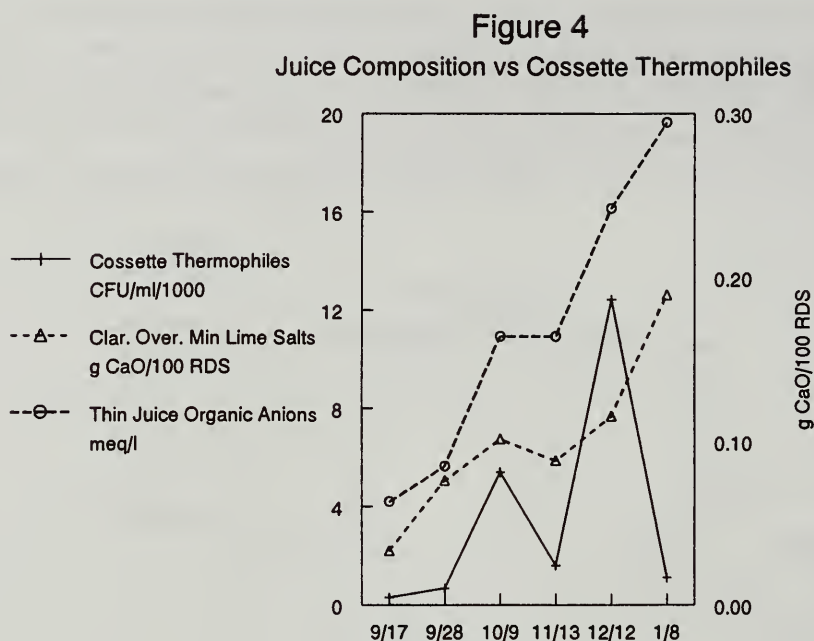
Note that the maximum flume water thermophile level of 50,000 CFU/ml on 12/12/01 corresponds with the highest thermophile level in cossettes (12,400 CFU/ml in laboratory extract) and that periods of low flume water thermophile activity (9/17, 9/28, and 1/8) correspond with low measured levels in cossettes. The samples collected on 10/9/01 and 11/13/01 do not particularly go along with the trend but when grab samples are collected from a continuous factory process there is always the possibility of differences due to non-homogeneous sample streams, lack of synchronization with the process flow, and other factors. Attempting to correlate levels of viable bacteria in different streams with each other brings up other possible complications suggested by the diffusion juice thermophile levels. Diffusion juice thermophiles are at peaks (especially in diffuser A) on 9/28/01 when cossette thermophiles are low and also do not reflect the high cossette thermophile levels on 12/12/01. Even though cossette thermophile levels are low on 9/28/01, microbiological growth within the diffuser could result in the higher levels observed in diffusion juice. Conversely, even though viable thermophilic bacteria in diffusion juice are low on 12/12/01, there is the possibility that the high level of cossette thermophiles enter the diffuser and multiply but do not survive diffuser conditions to appear in diffusion juice. Actually, even though high levels of thermophiles in cossettes and diffusion juice do not coincide, the levels in cossettes do appear to influence juice chemical composition. As will be shown, the high cossette thermophile level of 12/12/01 and, to a lesser extent, the peak in cossette thermophiles on 10/9/01 appear to be reflected in juice composition.

If diffusion juice total organic anions (data from Figure 1) are plotted alongside cossette thermophile levels there appears to be reasonably good correlation between the levels, as shown in Figure 3. Note that although diffusion juice organic anions show a general upward trend over the campaign, there are increases that coincide with the cossette thermophile high points on 10/9/01 and 12/12/01 and either decreases or periods of no change that coincide with decreases in cossette thermophiles.



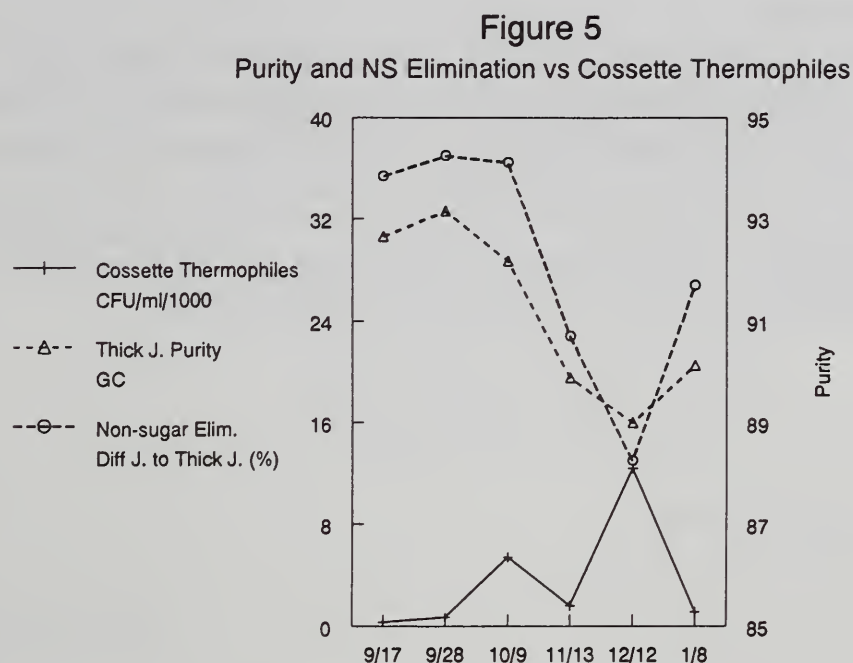
Diffusion juice organic anion composition, when the anions are not precipitated during liming and carbonation, influences the composition of juice streams after carbonation so one would expect clarifier overflow and thin juice characteristics to also coincide with the cossette thermophile levels. This appears to be the case, as shown in Figure 4, although as distance along the factory process stream from cossettes increases, the correlation becomes increasingly blurred.

Figure 4 shows several features in the plots of clarifier overflow minimum lime salts and thin juice total organic anions that appear to coincide with high cossette thermophiles, at least until the last set of samples (1/8/01). A complicating factor is the steady increase in diffusion juice invert from 0.5 g/100 RDS in the early campaign to nearly 1.0 g/100 RDS on 1/8/01. The destruction of increasing invert levels in liming and carbonation would produce gradually increasing chemically-produced organic anion levels superimposed on any increases due to microbiological growth.



The final and most important comparison of this study is involved with the question of whether or not the observed composition changes, that seem to be related to cossette thermophile levels, are reflected in final beet end purity and non-sugar elimination values. Figure 5 shows plots of thick juice purity determined by gas chromatography and non-sugar elimination calculated from an average diffusion juice GC purity (weighted for relative diffuser size) and thick juice purity. Both thick juice purity and non-sugar elimination show a general downward trend after the first several sample sets and both show the significant minimum value on 12/12/01, the date of maximum levels in beet flume thermophiles, cossette thermophiles, and raw juice organic anions.

Although not shown in figures, it should also be mentioned that several other analytical measurements reflect the extreme conditions present on 12/12/01. In particular, nearly all GC and pol purity values of the juice stream (clarifier overflow, 2nd carbonation, softener supply, thin juice, and thick juice) reached a low on that day and the sodium level increase (resulting from addition of soda ash to maintain alkalinity) was a maximum on December 12. All of this data provides strong evidence that the inoculation of cossettes with high-thermophile flume water produced chemical changes that were maintained through the sugar end.



SUMMARY

Periods of operating problems (high lime salts, lower thin juice purity, lower sugar extraction) in a beet sugar factory operating with a closed loop beet flume system were observed to be related to conditions (high organic loading) in the beet flume. During initial sample analysis in the 2000-01 campaign, several samples from the main factory stream (diffusion juice and thin juice) were found to contain detectable levels of butyrate, indicating that flume water had possibly been carried over into the factory juice stream. A more extensive series of tests were carried out during the 2001-02 campaign but during this period flume conditions were significantly better and no conclusive evidence of flume water carry over was found. However, several juice stream composition trends, particularly for total organic acids in diffusion juice, were found to correlate with cossette levels of thermophilic bacteria which, in turn, are related to thermophile levels in beet flume water.

ACKNOWLEDGMENTS

The author would like to thank Dennis Costesso of Amalgamated Research Inc., Alan Hieb, Shawn Bowen and Mike Fowers all of Amalgamated Sugar Co., for helpful suggestions. Sample collection and microbiological testing were also carried out by Mike Fowers. Organic anion determinations were made by Cheri McKay; gas chromatographic sucrose determinations by Diane Patterson; invert and cation analysis by Alla Bagramyan; and sample preparation as well as polarimetric purity determinations by Stephanie Olmstead, all of Amalgamated Research Inc.

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THE USE OF MEMBRANE FILTRATION TO REDUCE LIME USAGE IN PURIFICATION

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INTRODUCTION

Throughout the beet sugar industry there has been a drive to reduce the consumption of limestone both from a cost reduction viewpoint and increasingly due to environmental pressures on both supply and disposal. Within British Sugar the lime usage of the conventional process has been gradually reduced from 2% to 1.1% CaO on beet over the last 15 years. It is quite possible (but not yet proven in the factory) that lower levels could be achieved possibly down to about 0.8 % dependent on beet quality. Below that level the purity, colour and limesalts would become major issues and the nature of the mud become so changed that filtration would be impossible with current filtration.

In order to further reduce lime consumption we need to consider the use of alternative processes. One approach is to completely change the purification system as suggested by Amalgamated Research¹ in their chromatographic raw juice process or possibly eliminate it entirely by means of with raw juice crystallisation as demonstrated by Vaccari et al². Alternatively we can consider the major reactions of the conventional process and look for further reduction possibilities. These reactions can be considered to be

1. Precipitation of anions with insoluble or sparingly soluble calcium salts such as phosphate, citrate and oxalate.
2. Precipitation of magnesium
3. Precipitation and flocculation of high molecular weight colloidal compounds such as proteins and pectins etc
4. The destruction of invert sugars, which would otherwise lead to colour formation and pH drop during evaporation and crystallisation
5. Saponification of amides, principally glutamine, at high pH.
6. Microbial stabilisation of the juice

These reactions are due to a combination of the high temperature and pH in the process and the presence of calcium ions. Theoretically the precipitation reactions and elevation of juice pH to about pH11 only require about 0.2-0.3% CaO on beet. Additionally more lime is required in the form of calcium carbonate to aid settling and filtration of the precipitated colloidal material. There is also a significant colour removal effect due to adsorption of colour molecules onto the calcium carbonate crystals. This would suggest that up to a 70% further reduction in lime usage would be possible if a suitable process for removing the precipitated material could be found.

Several researchers ^{3,4} have suggested alternative purification processes along these lines using a pre-treatment with heating, alkali and/or flocculant addition (possibly with settling) followed by a membrane filtration stage to produce a juice stream for evaporation and crystallisation. The major difficulty in implementing these processes has been the high cost and unproven reliability of the membrane system on beet juice, together with the essential recovery of sugar from the waste streams of the process typically involving a clarifier underflow and the retentate from the membrane filtration. The clarifier underflow contains most of the materials that would normally end up in the conventional lime cake (beet particulates, soil, precipitated colloidal material etc) and is very difficult to filter or dewater. Usually diafiltration has been suggested for sugar recovery from the membrane retentate, which although being a possibility adds considerable cost to the process both in terms of increased membrane area and increased evaporation cost.

It is worth remembering that the overall target of any juice purification stage is to remove non-sugars that would otherwise affect the quality and recovery of sucrose and it would be difficult to justify any process that was not as efficient in cost terms as current processes. We consider that a realistic option for the future is to consider the addition of a membrane based purification process as a sidestream to a factory (to either increase overall capacity or to reduce lime consumption) with the recycle of waste streams to the main conventional process. This approach would enable an increase in throughput at minimal factory cost and very little extra lime usage.

PROCESS OVERVIEW

We have considered a process where a proportion of the main factory raw juice stream is passed through a pre-treatment and membrane filtration system before being mixed back into the main factory stream later in the process. For our testing we have mixed the membrane permeate back prior to the 2nd carbonatation stage, although other options have also been considered such as mixing to thin juice or using a separate evaporation stream and thick juice storage. The optimum system would be largely dependant on factory specific issues such as size of existing plant etc. Initially such a system would handle around 10 –30% of the factory flow giving an overall lime reduction of up to 20% for the whole factory.

The process is similar in concept to that proposed by Tecnimont⁵, which uses membrane filtration to effectively bypass the 1st carbonatation stage; although we consider there to be some advantages in retaining some degree of lime defecation.

An outline of the process is shown in Figure 1.

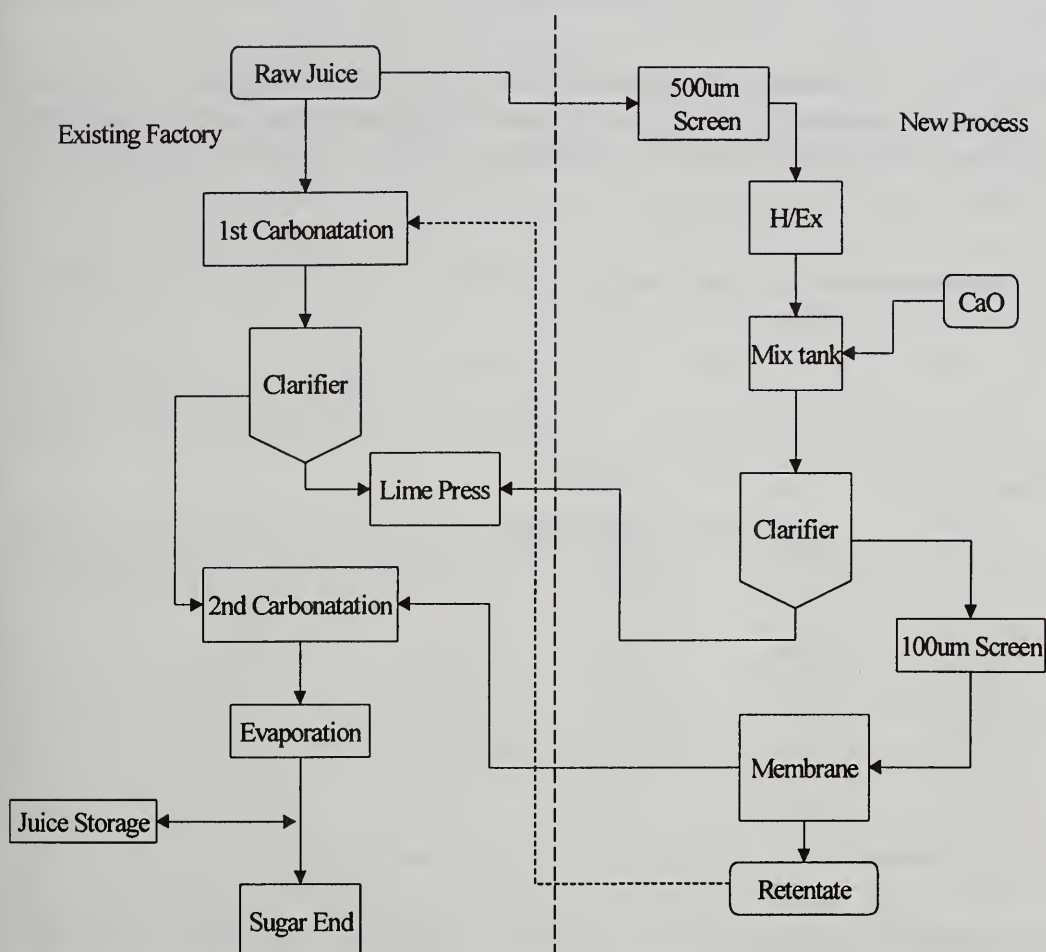


Figure 1. Process overview.

A proportion of the main factory raw juice flow is taken into the pre-treatment process where it is pre-screened on a 500um wedge wire screen to remove larger beet particulates. The juice is then heated to 85-90 C prior to the addition of lime in a small mixing tank (retention time 1-2 minutes). The combination of heating and rapid pH change causes coagulation of proteins and other colloidal material forming a precipitate, which is then settled in a clarifier with a retention time of 40-60minutes. The clarifier overflow is then screened through a 100 um filter mesh prior to membrane filtration. The screen is primarily there to protect the membrane system and catch any fibrous material carried through the clarifier, which could block the flow channels of the membrane system. The membrane permeate is then mixed back into the main factory stream prior to the 2nd carbonatation tank. The clarifier underflow is mixed with the concentrated 1st carbonatation slurry prior to the filter presses, (with some detrimental effects on filtration). The membrane retentate is recycled back to the 1st carbonatation stage of the main process. (It is worth noting at this point that British Sugar factories are operating a Dorr continuous defecocarbonatation system rather than a traditional preliming and main liming system.)

RESULTS

Juice Processing

The process has been tested on a combination of pilot and laboratory scale equipment. The pre-treatment and membrane filtration process has been carried out on a pilot scale system operating at 2 Tonnes/hr. Lime additions levels from 0.06 –0.3% CaO on juice were tested giving a pH range of 8.5 –12.

The solids balance of the pre-treatment process is shown in Table 1. Solids were measured by centrifugation and are expressed as % volume for volume.

Table 1. Solids balance across clarifier (% vol/vol).

Conditions	Raw Juice feed	Clarifier feed	Clarifier over flow	Clarifier under flow
Ca pH 8.5	1.5	2.1	0.5	33.0
Ca pH 9.5	1.5	2.2	0.4	30.6
Ca pH 10.0	1.4	2.3	0.4	34.5
Ca pH 10.5	1.5	2.6	0.6	25.6
Ca pH 11.0	1.5	3.0	1.4	21.4

As the pH of the juice is increased more suspended solids are produced in the feed to the clarifier. The suspended solids in the clarifier overflow remains at a consistent level below pH 10.5 at which point settling begins to deteriorate. Above pH 11 settling in the clarifier was very poor. The clarifier was operated at a ratio of 20:1 (overflow: underflow).

The membrane permeates from these experiments were analysed and the results shown in Table 2 compared to raw juice and a conventional carbonatated juice. Results are expressed as mg/kg on dry substance. (Solution colour was measured at 420nm after adjustment to pH 7 and filtration through a 0.45um filter).

Table 2. Analysis of membrane permeates.

Conditions	Concentration mg/kg on dry substance					Colour
	Sulphate	Oxalate	Phosphate	l - Lactate	Glucose	IU
Raw Juice	3366	3200	2543	1290	3226	9500
pH 8.5	2733	914	285	1806	2903	4205
pH 9.5	3069	888	201	1032	2839	4100
pH 10.0	3015	350	20	1548	2194	3700
pH 10.5	3501	167	0	2323	2258	3250
pH 11.0	2702	7	0	2258	1419	2700
2 nd carb juice	1554	0	0	3226	645	2000

The results show that at the higher pH operation the process can give similar elimination of oxalate and phosphate ions to the conventional process although levels of invert sugar are higher leading to increased juice colour and potentially further colour rise during evaporation.

In order to get the best compromise between juice quality and settling characteristics a pH of around 10 was chosen for further investigation (this equates to a lime usage of about 0.2% CaO on raw juice). Juice coagulated at pH 8.5 (0.06% CaO) was also used as this gives a greater potential lime reduction. Another factor to be considered here is the chemical stability of the membranes used. Operation at 85 – 90 C at higher pH would probably require the use of ceramic membranes whilst at a lower pH the use of cheaper polymeric membranes might be considered.

To investigate the effects of returning the membrane permeates back to the main process laboratory scale 2nd carbonatation tests were carried out with various mixtures of permeate and factory 1st carbonatation juice. The results are shown in Tables 3 and 4.

Table 3. Laboratory 2nd carbonatation tests (pH 10 Coagulated juice).

% Permeate	% 1 st carb juice	Purity	Colour	l-lactate g/l	glucose g/l	Filtration times
0	100	95.2	1655	0.361	0.010	3.8
10	90	94.2	1880	0.398	0.078	4.0
25	75	95.0	2030	0.557	0.149	3.2
50	50	94.6	2507	0.515	0.180	3.5
100	0	94.6	2945	0.420	0.350	3.9

Table 4. Laboratory 2nd carbonatation tests (pH 8.5 Coagulated juice).

% Permeate	% 1 st carb juice	Purity	Colour	l-lactate g/l	glucose g/l	Filtration times
0	100	94.5	1655	0.361	0.010	3.8
10	90	94.0	1900	0.420	0.800	4.0
25	75	95.0	2150	0.512	0.160	4.0
50	50	93.5	2850	0.515	0.220	3.5
100	0	93.8	4212	0.430	0.450	3.9

Note: Filtration times are for filtration of 200ml of juice through a 47mm diameter 8 um filter.

At both pH's mixing back the membrane permeate had no effect on 2nd carbonatation filtration times although at the lower pH there is some reduction in purity at high permeate addition levels. There is also some increase in juice colour under both conditions largely due to the higher levels of invert sugars in the membrane permeates. Overall the results suggest that the membrane permeate could be processed separately and mixed back into the main factory stream at a later point. Larger quantities of juice were mixed and gassed down in batches to simulate 2nd carbonatation. These were then softened and evaporated prior to crystallisation in a pilot scale vacuum pan. Sugar colours of below 30 IU were achievable from 25% mixtures of permeate at both pH 8.5 and 10. Crystallisations of 100% permeate juices produced sugars of 50-100 IU colour.

Waste stream processing

It is proposed that the clarifier underflow from the pre-treatment process could be mixed back to the thickened 1st carbonatation mud of the main process prior to the filter presses (or rotary vacuum filters). Laboratory filtration tests were carried out on mixtures to test this.

The results are shown in Figure 2 where the results are expressed as relative filtration times against the % of flow through the membrane system (% sidestream). The results are similar for both pH levels tested showing that the incorporation of clarifier underflow reduces the filterability of carbonatation mud. Increased filtration area of about 50% would be required for a 25% sidestream although for larger flow an alternative process may be required. One alternative option considered is the use of a 2-stage decanter centrifuge system with addition of dilution water between the 2 stages. The concentrates produced were found to be of suitable quality to recycle to the clarifier overflow and the mud produced was of a similar consistency to conventional limesludge and could be mixed with it.

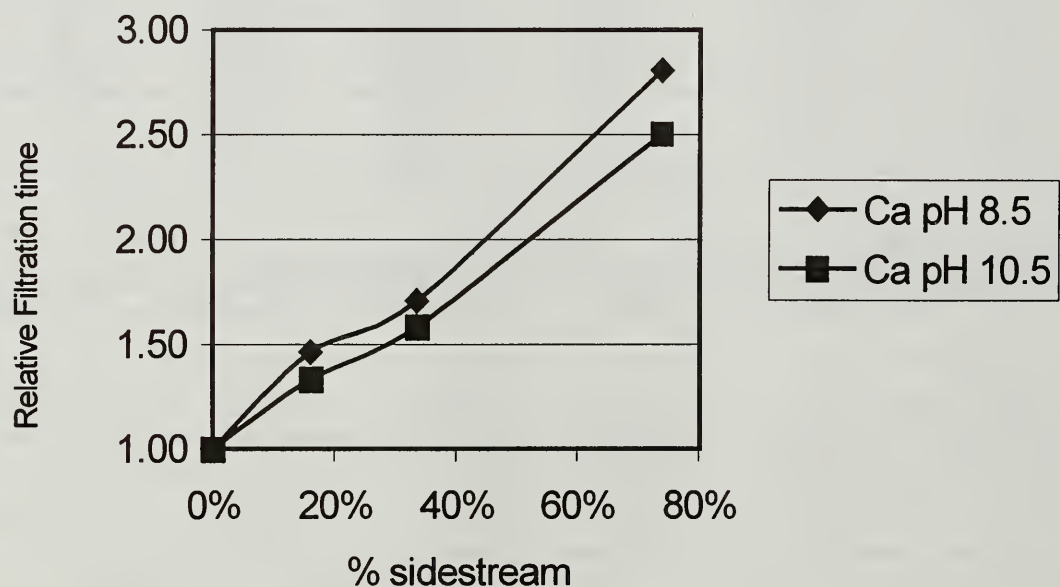


Figure 2. Effect of clarifier underflow on filtration of carbonatation mud.

Figure 3 shows the effect of the amount of water addition (expressed as % beet sliced) on the sugar loss in the mud. (The numbers are based on a 100% membrane stream rather than a sidestream).

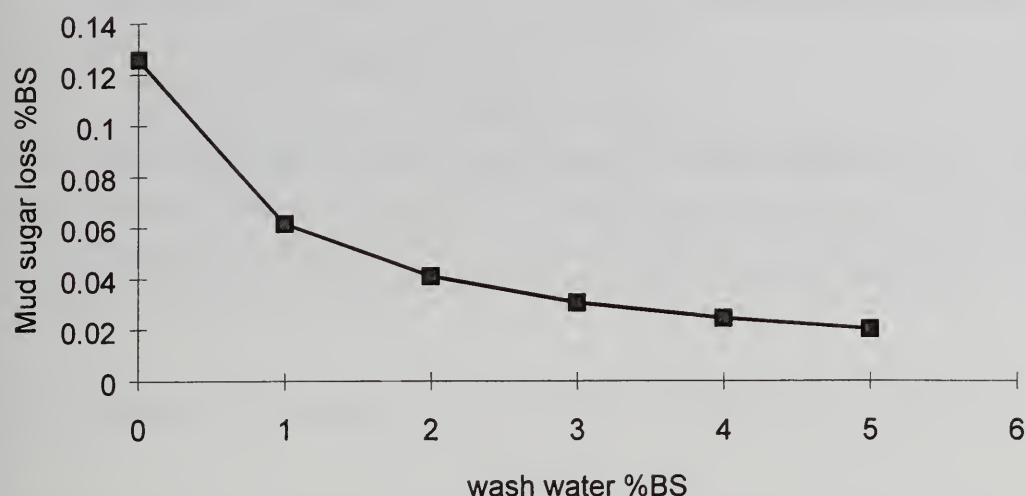


Figure 3. Sugar loss vs. water usage for 2 stage decanter centrifuge system.

The results show that reasonable sugar losses of about 0.03% sugar on beet sliced could be achieved with wash water addition of 4% on beet sliced, although with the added cost of the centrifuges. A single stage decanter centrifuge might be an option if higher sugar losses were acceptable.

The suggested process for handling the membrane retentate is to recycle it back to the main stream prior to the whole carbonatation process. Mixtures of membrane retentate and raw juice were purified using a laboratory scale carbonatation procedure to test this. The results are given in Table 5 compared to a purification of a control raw juice.

Table 5. Laboratory carbonatation tests on membrane retentate mixtures.

Sample	Purity	Colour IU	Lactic acid g/l	Glucose g/l	Filtration times
Control	94.8	1055	0.361	0.010	3.8
5% pH 8.5	94.7	1033	0.410	0.012	3.3
10% pH 8.5	94.6	1102	0.659	0.024	3.1
20% pH 8.5	94.9	1137	0.502	0.010	3.5
5% pH10.5	94.8	1070	0.495	0.012	3.5
10% pH 10.5	94.7	1095	0.480	0.015	4.0
20% pH 10.5	94.6	1194	0.450	0.015	3.8

Note

5% recycle of retentate is equivalent to running a 20% sidestream at a 5X concentration factor.
 10% recycle of retentate is equivalent to running a 35% sidestream at a 5X concentration factor.
 20% recycle of retentate is equivalent to running a 55% sidestream at a 5X concentration factor.

Results show that mixing retentate (5X) back to raw juice would have no serious effect on carbonation performance

CONCLUSIONS

Our research has shown that a realistic option for the future is to consider the addition of a membrane based purification process as a sidestream to a factory to either increase overall capacity or reduce lime consumption. A system operating at 25% of factory throughput could give a lime reduction of up to 20% for the whole factory. Advantages of taking this approach include:

Similar purity thin juice compared to conventional process with sugar colour less than 30 IU achievable.

Simplified Handling of Waste Streams. Operating as a sidestream enables the waste streams from the process to be recycled back to the main factory stream reducing the need for expensive additional processing equipment.

Reduced Size of Membrane Plant. Operating at this scale would enable membranes to be used at around 5x concentration factor (ratio of feed to retentate flow). This allows a wider choice of membrane type and also gives higher average flux rates than would otherwise be achievable reducing the amount of membrane area required.

Reduction in Risk of Project. Only a proportion of the factory flow is dependant on the membranes, which still need to be proven on a large scale in the beet sugar industry.

Potential for Future Expansion. The membrane systems are essentially a modular design allowing for future increase in capacity of the sidestream (and hence reductions in lime usage) as confidence in the system is developed. Although further decolourisation or possibly the use of cooling crystallisation may be required to produce suitable quality sugar if the process is expanded further.

Facilitation of Process Development. Production of waste streams in large quantities allowing for testing of alternative options for handling these streams, which will be needed, if the membrane plant is expanded in future. It is often difficult to produce suitable quantities of the waste streams (particularly retentate) for processing when testing on a smaller scale.

Overall, our research would suggest that this type of process might be a suitable first step for introducing membranes into the beet sugar industry on a production scale, giving initial benefits of lime reduction together with building confidence in the reliability of membranes, potentially allowing other processes to be developed in the future.

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STEAM DRYING OF BEET PULP AND BAGASSE

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INTRODUCTION

Drying of beet pulp has so far normally been done in directly fired rotating drums. It is strange that it has taken so long time to find good alternatives to this technology, especially because the sugar industry has been good at learning to economise through its long history. When you consider what is done in rotating drum dryers, it is a severe waste of exergy. Exergy-wise, it is on level with the processing of the time, when juice was concentrated in open pans with a fire underneath.

The drum drying has furthermore a pollution problem as the plume of white vapour is often followed by a brown haze, which represents more or less burned fine particles. It also gives a bad smell. Finally, drum dryers cause a product loss somewhere between 3 and 15% depending on temperature and type of dryers.

The drying process is:



Why not:



That is what you do, when you press the pulp. But the conventional presses produce only a little over the 30 % DS.

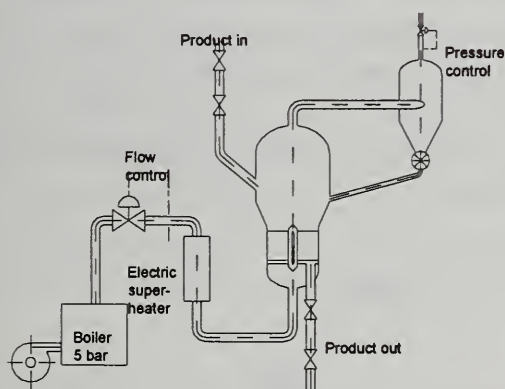
There have been some interesting studies at Südzucker, Germany followed by a pilot press installed at the sugar factory in Regensburg. The technology was based on distributing the pulp in thin layers between draining cloth, long pressing time, and a relative high pressure. The pressed pulp came out with 50% DS looking like humid cardboard. The energy consumption was fair, but the mechanical problems could not be overcome.

Keeping in mind that the final result should be dried pulp and water, you should be happy if the vapour could be turned into heat at a high exergy level. The drum drying does not

fulfill this wish, as the vapour only can be condensed at temperatures around that temperature level, that in a sugar factory is worthless. If the drying could be done in superheated steam without air at a pressure, that fits for recovery in a sugar factory you get indirectly to the formula (B). You will use a lot of energy, but you get it fully back. It will be as done by each step of your juice evaporators. That was the hoped for result of a development that was started at the Danish Sugar Factories (Danisco).

The Development

The goal was drying in superheated steam under a pressure that fit to supply the juice evaporation. As the oil prices went up in 1981 there was allocated money to the project. All possibilities were open. There were no ideas about which form it would take. So the nature of the drying in steam had to be examined down to its basics. In a small chamber, samples of pulp were dried at various pressure and temperatures in a fixed bed. After doing the first basic examinations, a pressurised fluid bed was established (Figure 1). Steam from a boiler was blown once through. It was not possible to produce nice dried pulp, but information about needed retention time was gained, and k-values from inserted heating plates were measured. Then one of the first conclusions was obtained.



Drying was not possible at the wanted pressure in a fixed bed with the pulp laying on a belt through which steam was blown down through (Figure 2). The drying time would then be so long, that the Maillard reaction would colour the product dark brown or black.

Figure 1. Lab scale pressurised fluid bed.

It was also clear that drying in heated vertical tubes in which the pulp is blown up and down was not a realistic process. This method would not make it possible to get the needed drying time of at least 1 minute for normal pulp (Figure 3). The effort was therefore concentrated on the fluid bed technology.

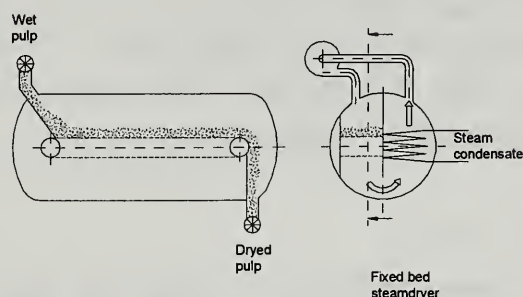
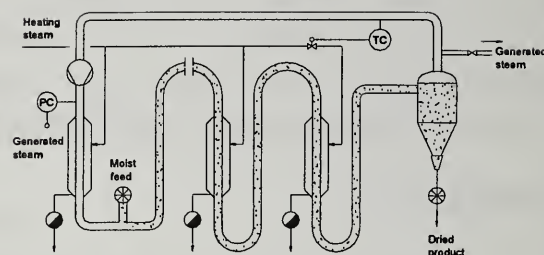


Figure 2. Fixed bed steamdryer.

Figure 3. Fast flow steamdryer.
(Exergy Teknik, Sweden)

The next problem that occurred, was that beet pulp could not form a stable fluid bed. A lot of lab models with air as the fluidising medium were built, combined with theoretical studies in order to find a form of bed in which all the particles would keep moving without blowing too many particles out through the top of the bed. Dummies for heating plates was put in to find how many sq ft of surface could be placed in a given size of fluid bed. From measured k-values, the possible heat transfer could be calculated. It showed that only a small part of the heat could be transferred by inserted heating panels. Increasing the size of the fluid bed to get enough space for the heating plates, causes the retention time to be so large that the Maillard reaction would be a problem. The fluid bed should therefore be designed to include a large circulation of superheated steam to be reheated, before it returns to the fluid bed. It took 2 years with these basic theoretical and experimental studies until it became the time to build a larger pilot plant.

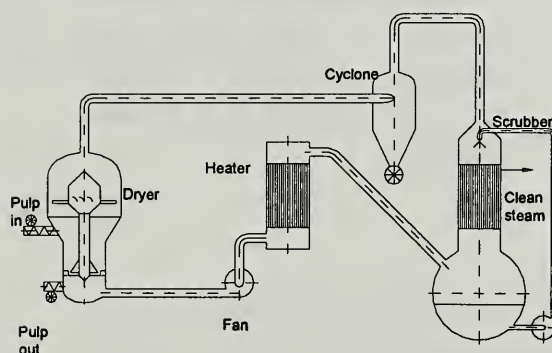


Figure 4 Pilot plant

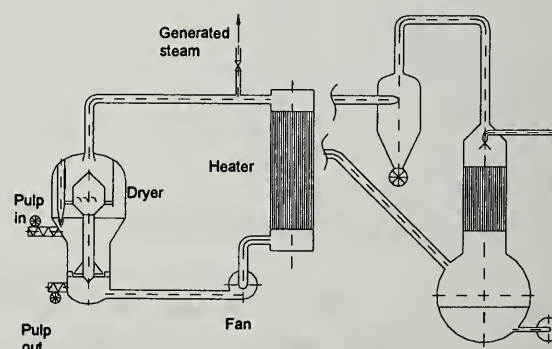


Figure 5 Modified pilot plant

The pilot plant was built with financial support from the EU. The pilot plant is seen in Figure 4. A large flow of superheated steam is circulated by a fan up through the dryer. After the dryer, dust is separated in a cyclone before the steam is scrubbed and reheated and then returned to the fluid bed by the fan. The installation was good for all kinds of measurements, and in stages various functions were modified. The most important modification was to build in a dust separation that was so good that the external dust

separation could be omitted. The pilot plant was modified into what seen in Figure 5. Then the way was open to build a prototype, where the heat exchanger was placed in the center, and the fan built into the bottom of the dryer itself. In this way everything could be included in one pressure vessel.

A prototype was built, and after a couple of years with problems and rebuilding, a stable function was achieved with a water evaporation of 8,7 t/h. It was then presented by Arne S Jensen at the international sugar conference, CITS, in Ferrara in Italy in 1987. Many visitors came to see the installation at Stege Sugar in Denmark. Among them were also the engineers from a French sugar factory Lesaffre Frères SA, in Nangis in France. They decided to build such a dryer, but for 25 t/h evaporation. A quotation was made and the dryer was delivered by Danisco and designed by the team at Stege Sugar Factory, that had designed the prototype. This first larger dryer, size 8, was brought to successful operation in 1990.

Arne Sloth Jensen went then with the technology to NIRO A/S (a daughter company of Danisco at that time). During the years at NIRO, a larger dryer, a size 10, was designed. In that period 7 factories in Europe had steamdryers installed, for a total of 8 factories. But in 1997 NIRO A/S decided to stop the development, and Arne Sloth Jensen left NIRO A/S.

The Niro Steamdryers loaded to more than 2/3 of the rated capacity had difficulties to operate stably. Furthermore many of the factories now wanted more than the rated capacity. That task was taken up by Arne Sloth Jensen and the company EnerDry.

One problem was that the high loaded fluid bed got stopped now and then and the job to clean it took several hours. It was necessary to improve the fluidisation. The perforated bottom plate was rebuilt in several steps to a new design of a curved bottom. The distribution of the holes in the perforated plates was changed and some jet effect was added.

In order to get a higher capacity, the dust separation in the top was rebuilt, so that a larger circulation of steam within the dryer was possible without having wet particles blown into the dust separation. The larger circulation then demanded a larger fan and motor. Up to 25% more capacity was obtained.

It was very soon clear, that the dividing of the fluid bed in high vertical cells would be a hindrance for the increased capacity. The division in high vertical cells was the main claim in the patent for the old dryer. Thereby a new dryer was created.

Five of the 8 sugar factories in Europe with steamdrying of pulp have now had their dryer rebuilt to the new specification.

How The New Steamdryer Works

The pulp is fed through the rotary valve (1) to the screw (2) that brings the pulp into the pressure vessel (3) filled with superheated steam. The only moving part in the dryer is the impeller (4) circulating this steam up through the perforated curved bottom (5) into a low ring-shaped fluid bed (6) where the pulp is kept "fluid" swirling around as the arrows indicate. Guiding plates (not shown) make the pulp move forward in the ring to the discharge screw (8) and subsequently out through the rotary valve (9).

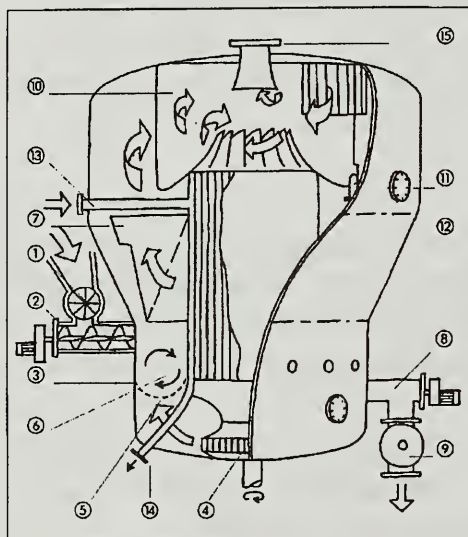


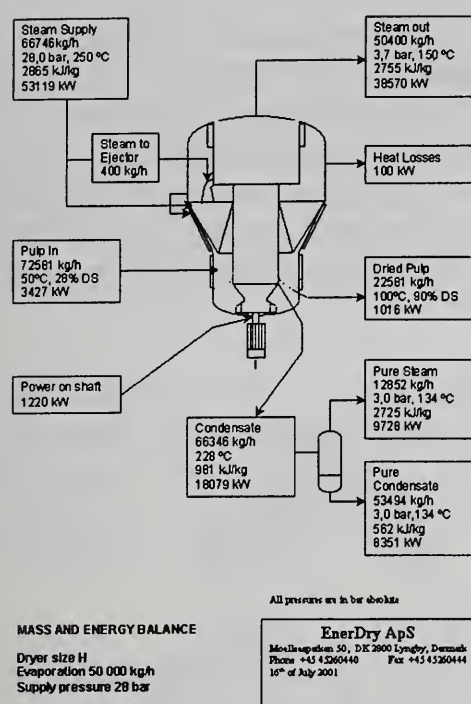
Figure 6. A look into the new dryer.

The lighter particles are blown up between the plates (7) radiating from the heat exchanger (12) outwards towards the conical vessel wall without reaching this. Due to the reduced velocity the particles fall onto the forward inclined plates, slide down on those, and pass the gap between the plates and the conical vessel wall. This way also the lighter particles pass forward around the dryer and arrive at the discharge.

The circulating steam arrives into the upper part of the dryer, where dust is separated in the main cyclone (10). By means of an ejector the dust passes out through the pipe (11) and goes out with the dried product.

The dust-free steam moves downwards inside the tubes in the heat exchanger (12) where it is reheated, as steam at elevated pressure is supplied through the pipe (13). The supply steam is condensed and leaves the dryer through the pipe (14). With a higher supply steam pressure, a higher temperature of the circulating steam is achieved, and this again increases the drying potential of the steam. Thus, the capacity of the dryer is dependent upon supplied steam pressure.

The steam that has evaporated from the pulp leaves the dryer through the pipe (15) right in the center of the vortex. That gives a steam so dust free that it can be condensed between pipes in a tube bundle. A small quantity of dust (2 to 10 ppm) will be flushed out with the condensate. Using this steam, and the energy in the hot condensate leaving the dryer through the pipe (14) almost 100% energy recovery is achieved.

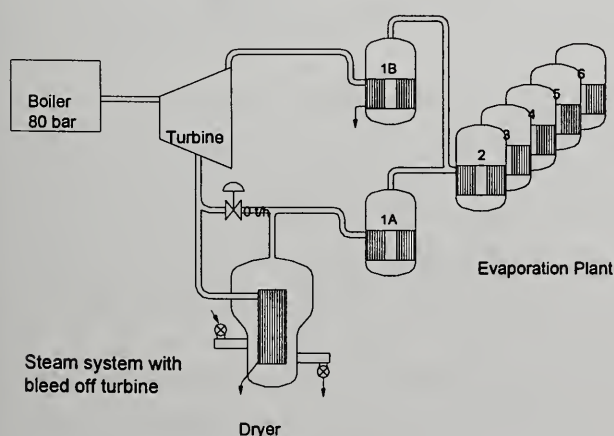


Drying under pressure requires pulp to be fed into the pressure system and out again. This is done through rotary valves. They have a design originally developed 40 years ago in the Swedish paper pulp industry, where they were used for feeding wood chips into the cookers having 7 to 12 bars. That was also feeding into steam under pressure.

Figure 7. Mass and energy balance for a size H dryer.

The largest dryer is a size H. It has a guaranteed capacity of 50 t/h evaporation when supplied with 27 bar g (390 psig) steam. After the positive experience with the rebuilding by EnerDry of a large Niro dryer in Holland ready for the 2001 campaign, it is most likely that a new size H will reach 55 t/h evaporation. The dryer can also be operated with a supply steam with much lower pressure, e.i. 200 psig. However, that will reduce the guaranteed evaporation capacity to 34 t/h.

How Does the Steamdryer Fit into the Steam System in an Existing Sugar Factory?



The classic steam system in a sugar factory with high pressure boilers and steamdrying is shown in Figure 8. If a high power production is needed, this is the way to do it, if a new turbine is installed.

Figure 8. Ideal system for high power production.

It is often said that it is complicated and difficult to fit steamdrying into an existing sugar factory. It shall be pointed out, that it is *never* necessary to do changes in the boiler house. Changes on turbines are not necessary either, but it might be an advantage. It will be necessary that the first step of the evaporator consist of 2 bodies, so one can be used for steam from the steamdryer, and the other for the steam from the turbine, in order not to mix impurities in the boiler feed water. If there is no suitable evaporator to receive the steam from the steamdryer this might be the opportunity to add more surface to the first step of the evaporator and thereby bring the pressure/temperature down or by adding a step in front to reduce the condenser losses.

In existing factories it should be studied on case by case basis how the integration of the steamdrying should be made. If you have high pressure boilers, and no steam outtake on the turbine, a proposal could be to supply the dryer through a small turbine that brings the pressure down to the pressure needed for the dryer. At the inlet of the turbine you regulate the pressure to the dryer, and the turbine can drive a power generator, which is coupled to the grid in the factory. Please see Figure 9.

If the size of a sugar factory is increased, it might be necessary to more boiler capacity. If there is a high pressure boiler you could buy a low pressure boiler to supply the dryer, and agree to buy power if your own production is too small. Please see figure 10.

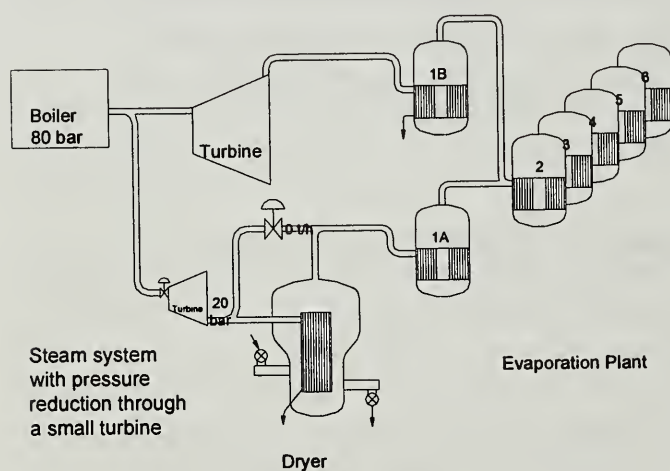


Figure 9.

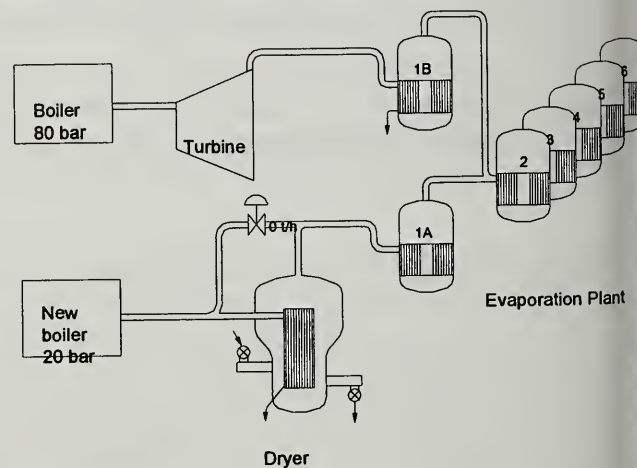


Figure10.

When you introduce steamdrying, you will normally save about all the fuel needed for the alternative drum drying. The possibilities for power production are in general reduced. You do not lose any energy. You only lose the possibility to transform a part of this energy into power. Any fuel savings will reduce your possibility to produce power. If you save fuel by optimising the evaporation and the sugar house, you will lose relatively more power production than by introducing steamdrying. Please see the A.S. Jensen paper from ASSBT in Orlando 1999.

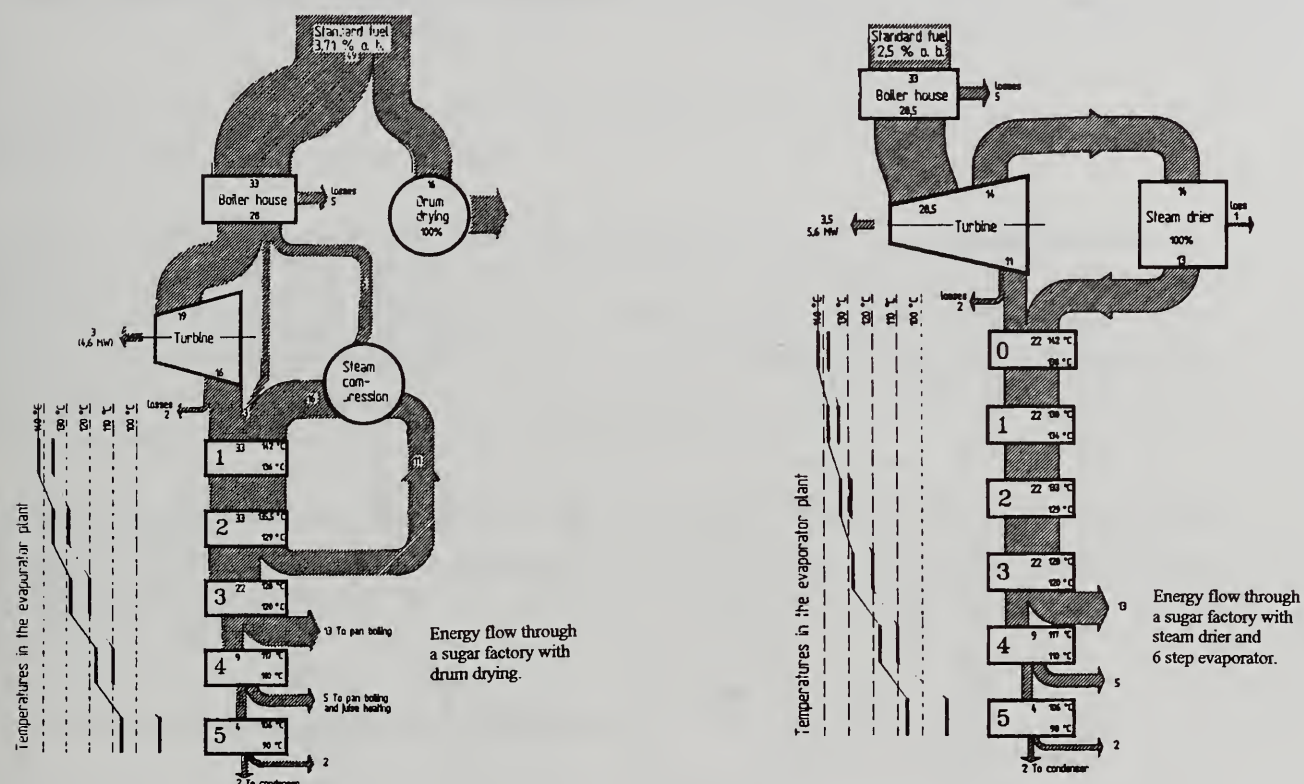


Figure 11. Example of energy flow with drum drying and steamdrying.

In Figure 11 we see an example of energy flow in a factory with drum drying and after introduction of steamdrying. The “width of the flow” illustrates the energy flow, also indicated by numbers for the amount of standard steam in percent of beets. In this case, the turbine has been exchanged for a turbine with an outtake to supply the steamdrier. The evaporation has got one step more, and the steam compression has been stopped. The result is that all the fuel for the drum drying is saved. The boiler house produces the same amount of steam as before and there is even produced more power. It should be mentioned, that introducing of steamdrying will never in itself increase power production. This example illustrates that you often can make other improvements. Here is illustrated, that you should rather have more steps in your evaporations then use live steam for steam compression, if you want to improve the power production.

Pulp. The Future Bio Fuel?

An alternative is to use the pulp as fuel. If all pulp is steam dried and 75% of the pulp is fired in the boilers, there will be energy enough for a reasonably energy economic factory.

The pulp is a good bio fuel. When dried the lower heat value is approximately 17,000 kJ/kg. That is on the level of dried lignite. But the sand content is lower, and the beet pulp is so to say a “washed” fuel, as it has passed the extraction. Thereby the content of K and Na and other salts has been brought down. That indicates that the pulp should be a quite good fuel even for boilers producing high pressure steam. It could be fired with a spreader stoker and burned out on a moving grate or a cooled vibrating grate. Many boilers could be rebuilt for firing dried beet pulp.

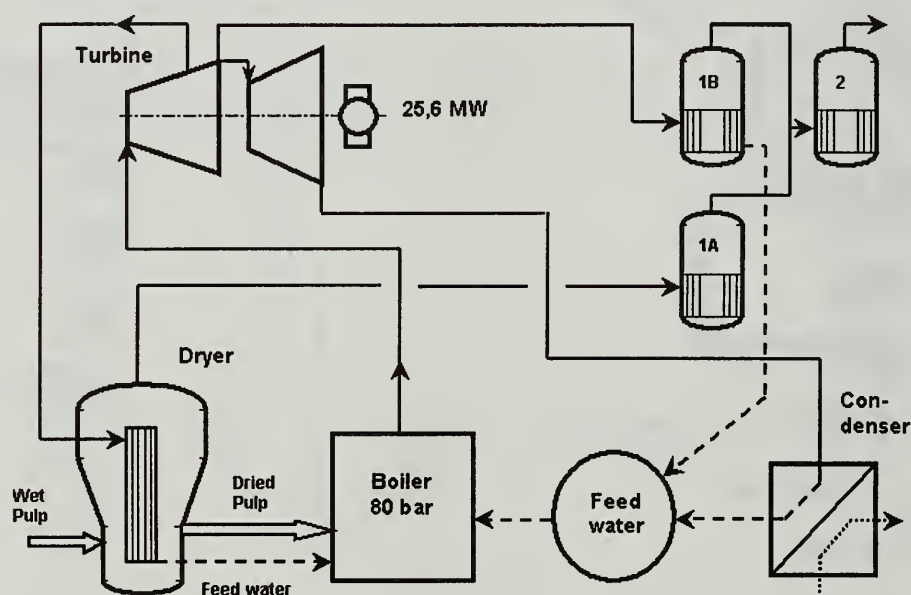


Figure 12. Steam system in a sugar factory combusting all the beet pulp

Figure 12 shows how the steam system in the factory could be. All the pressed pulp is steam dried and burned in the boilers. That gives a surplus of steam. Therefore the turbine has a low pressure part bringing this steam down to moderate vacuum. A sugar factory processing 12,000 t beets/24 h will be able to produce 25,6 MW_e without supplementary fuel.

This is based on the following data:

12 000 t beets / 24 h
Dried pulp: 5,53 % on beet.
Steam to evap. (1A + 1B): 22% on beets.
Pulp is pressed to 28 % DS.
Live steam: 80 bar 520°C.
Steam to dryers: 22 bar.
Vacuum: 0,2 bar (60 °C)
Efficiencies: Boiler 90%
 Turbine 88%
 Generator 95%

For the time being, the price for pulp pellets is still such, that it pays to buy fuel and sell the pellets. But the situation may change.

Beet pulp is considered as a bio fuel. That means, that the CO₂ emissions from the combustion will not be taxed, as the CO₂ is neutralized by the reverse process the same year. A tax on CO₂ emissions from fossil fuels can change the economical balance. There could also come a direct obligation to reduce the CO₂ emission. First of all, steamdrying in itself will reduce the CO₂ emissions by 33%. Burning the pulp is then the next step, and thereby the fossil CO₂ emissions can be reduced to 0.

It has become more and more common in many countries, that the big power companies are obliged to have a certain part of the electric power produced as “green” power. Power production according to Figure 12 is green power production. Cooperation with a power company may be possible.

Power Production from Sugar Cane Bagasse

Cane sugar mills normally burn their bagasse in boilers. There are so many fibres in the cane, that it is easy to have enough steam for the factory. The bagasse has around 50% DS. Burning this is using primary energy to evaporate the water in the bagasse. Furthermore, all the steam arising thereby takes up volume in the combustion chamber and all the way out through the stack. This limits the capacity of the boiler, and reduces the combustion temperature. The capacity of the boilers are therefore low compared to their physical size, and it is more difficult to produce steam of high pressure and temperature.

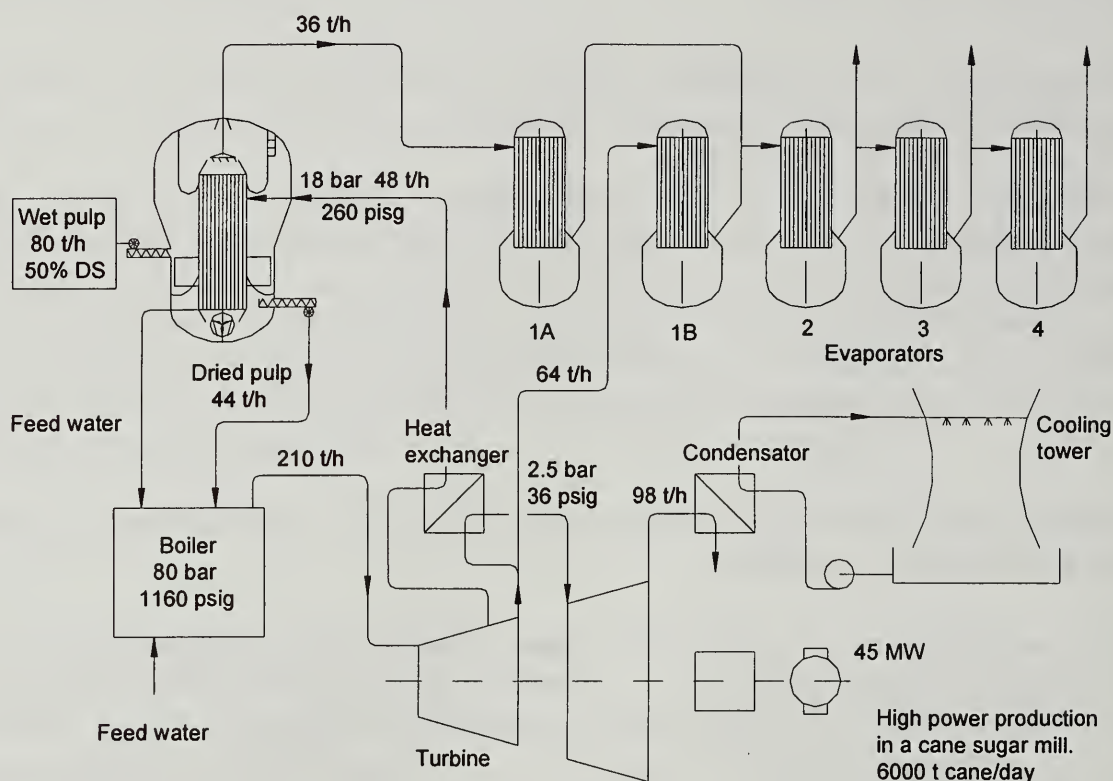


Figure 13. High power production in a cane sugar mill.

If the bagasse could be dried by steamdrying before combustion, it would be possible to produce a large co-generation of power. Figure 13 illustrates how the possible large steam production can be used. There will be more steam than necessary for the factory. Therefore a part shall pass the turbine all the way down through the turbine to vacuum. A cooling tower is necessary. The example is based on a crushing of 6000 t/day and it is assumed that the mill train is electrified. Out of the power production of 45 MW, a portion is used by the mill itself.

CONCLUSION

Steamdrying is now available in an improved form with larger capacity in a more compact dryer, thereby cheaper than ever. The operation is now also stable at high capacity. Steamdrying can make a beet sugar factory self-sufficient in fuel as well as in the cane sugar industry. Steamdrying of bagasse can give a high efficiency in co-generation of power in a cane sugar mill.

The steamdrying technology offers:

Near 100 % energy saving compared to drum drying.

No air pollution.

No product loss.

Simple to operate from a central control room.

Winner of First Margaret Clarke Best Paper Award

**INDUSTRIAL ECONOMICAL OPTIMIZATION OF THE JUICE
EXTRACTION PROCESS FOR SPANISH AUTUMN AND
SPRING SOWN BEETS**

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ABSTRACT

Results obtained in an extensive study on optimization of juice extraction are presented.

Over a period of more than two years, processing of about 400 beet samples at pilot plant scale has given us a very high number of very different results related to the special and typical conditions of the Spanish raw material. Before beginning this R&D project, we had considered the very important variability of harvested beets in Spain of special interest for knowing much more about a real optimization of the diffusion process.

We had considered that only by large variations of the general basic conditions, would we be able to design a simulation experiment at pilot plant scale which would be representative of the industrial process. Spanish beet sugar production offers unique conditions regarding the well known spring sown beets and the special autumn sown beets.

During the 2002 CITS meeting in Paris, we presented the developed pilot plant methodology as well as the first results related to the spring sown beets. The following units compose the pilot plant (from beet to thick juice): beet washer, slicing machine, diffuser (DDS type), juice purification (lab scale) and evapo-juice concentration.

The main objective of this project was optimization of the extraction process according to the following parameters:

- Sugar losses
- Non sugar extraction
- Juice draft and energy economy
- Color precursor and formation balance
- Effective alkalinity and soda ash consumption
- Molasses sugar losses

The results are based on the following main variables:

- Southern and northern Spanish beet composition
- Cossette quality
- Degree of extraction oxygenation
- Cossette exhaustion
- Process temperature

The project now concluded provides us a direct economical and profitability view about the optimal industrial diffusion plant parameters. The sensitivity to these principal parameters is very much higher in the southern beet sugar production than in the other northern basic conditions.

DESCRIPTION OF THE PILOT PLANT EQUIPMENT

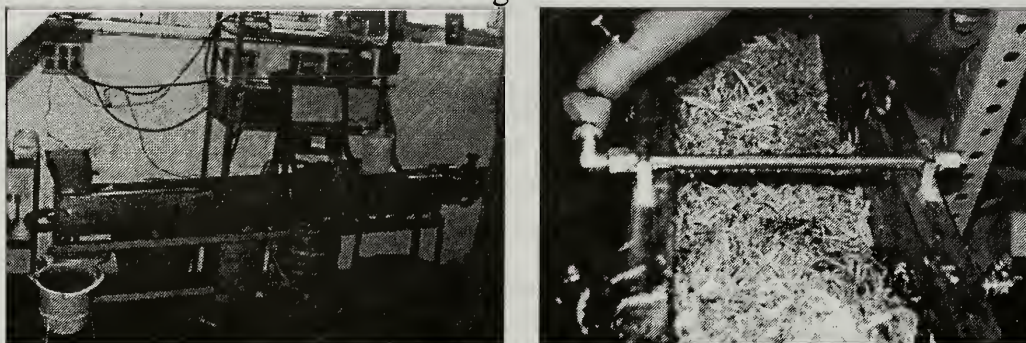
The pilot plant was composed of the following elements:

- Cold storage room
- Beet washer
- Slicing machine
- Diffuser (DDS type)
- Juice purification
- Evapo-juice concentration

Diffuser DDS with mechanism for flow, temperature and residence time control.

- Capacity: 20 Kg/h.
- 60 to 90 min residence time
-

Figure 1



Juice purification and evapo-juice concentration were done on a laboratory scale (batch process). The juice purification parameters were:

- Preliming: 20 min. 55 °C
- Main liming: 100 % CaO, 30 min. , 85 °C
- First carbonation pH:11,0–11,2, 85 °C, 20 min.
- Second carbonation pH 8,8–9,2, 95 °C, 15 min.

Figure 2



The juice concentration was done to 68 Brix under reduced pressure. Finally there was a heat treatment at 120 °C, 5 min, in the autoclave to reproduce evaporation conditions.

METHODOLOGY

The operation methodology was the same for all experimentation. The followig experiments were planned to study the juice extraction optimization:

Northern Spanish beet variables

- Juice Draft (100, 115, 130, 140% o.b.)
- Oxygenation degree (anaerobic or aerobic process)
- Cossette quality. A (Silene number 18, mush < 1%), B (mush content 50%), C “combs” 50%).
- Process temperature (65, 72, 80 °C)

Southern Spanish beet variables

- Juice Draft (65, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135 % o.b.)
- Process temperature (66-67, 71-72, 76-77 °C)
- Beet composition (high and low beet quality).
- Water press return (high and low beet quality).

Once the operating conditions were fixed and stable, the following parameters were determined:

- Sugar losses
- Non sugar extraction
- Juice draft
- Color formation
- Soda ash consumption
- Molasses sugar losses
- Calculation of global sugar yield.

Other specific parameters were measured (reducing sugars, amides, organic acids, etc.) using analytical methods found in literature.

To estimate the total sugar losses we took into account the pulp sugar losses, the sugar in molasses and the sugar losses in molasses due to NaOH addition in the process. Another critical value to optimize juice extraction process was the thick juice color because the two thick juice parameters (purity and color) are responsible for the white sugar quality and sugar production costs.

To calculate the sugar in molasses we calculated the non sugars content in thick juice and assumed a molasses purity of 59, which was the average in the last northern campaign. We also took into account the sugar losses in molasses due to NaOH addition. We used the melassigenic coefficient of NaOH from Silin (4.61). The NaOH consumption was calculated from the effective alkalinity.

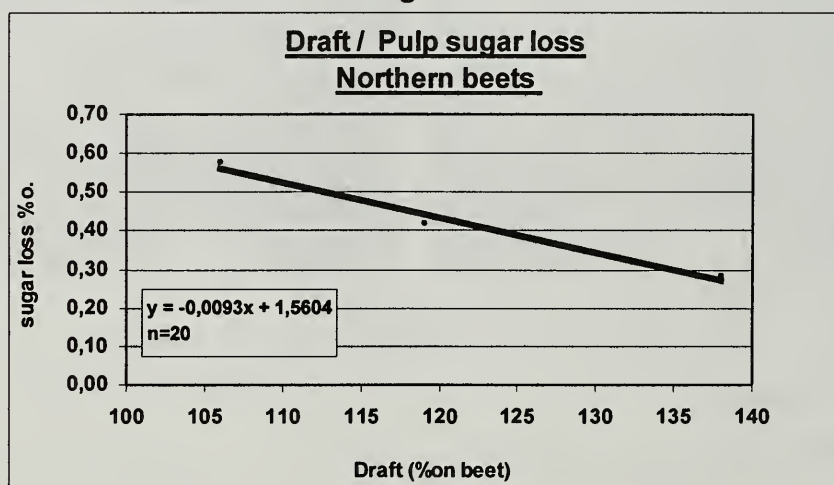
Finally a statistical treatment of the different parameters analyzed was done. For every experiment, the average value and standard deviation for every parameter was determined. The r^2 correlation is shown from data obtained for each of the different operating conditions, to conclude whether the studied variables have any influence on the results.

RESULTS

Cossette Exhaustion

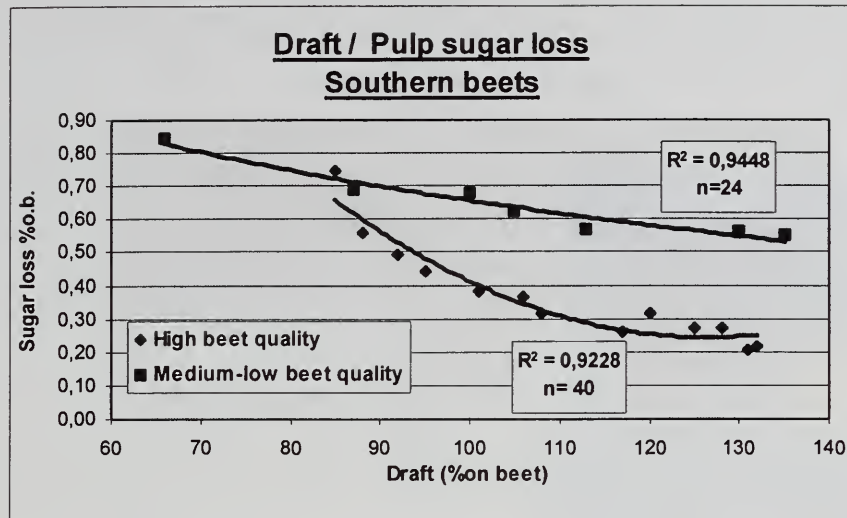
With the pilot plant we were able to do the complete process from beet to thick juice. We kept constant extraction temperature, with cossette quality being the only variable the juice draft. The draft was calculated by weight. At higher draft, sugar loss in pulp decreased, as shown in Figure 3 for northern beets.

Figure 3



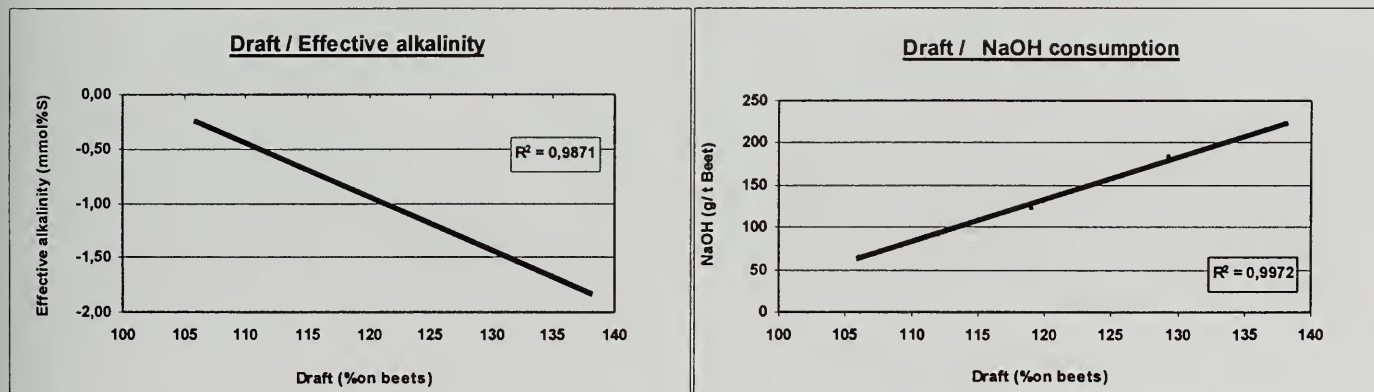
We obtained the same result with southern beets (at higher draft, lower pulp sugar losses) but with a different trend for different beet composition (high and medium-low beet quality) (Figure 4.)

Figure 4



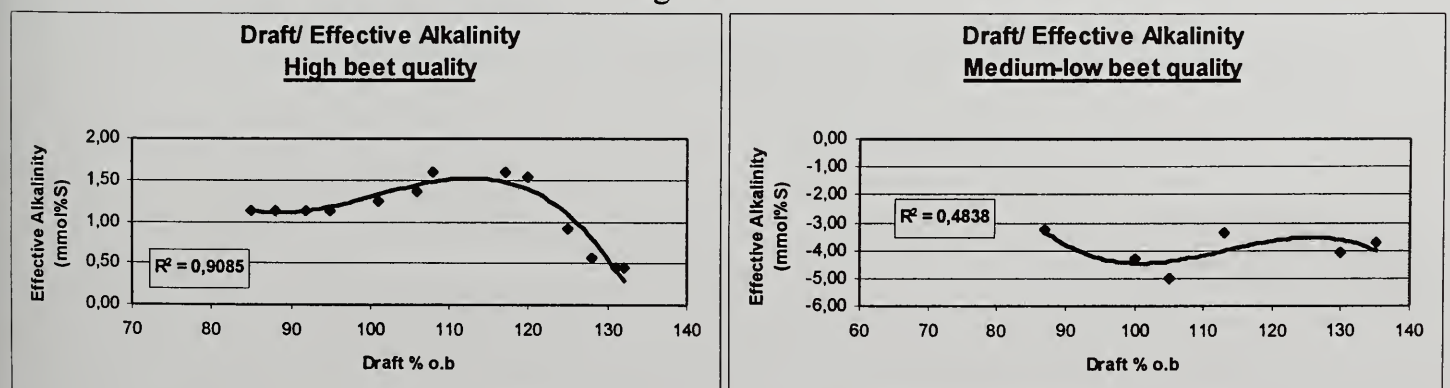
Also the lower juice draft would reduce the volume of juice to be evaporated with a substantial fuel economy. With northern beets, an increase in the draft from 105 to 125% will triple the NaOH consumption. The effective alkalinity decreases as the draft increases (Figure 5).

Figure 5



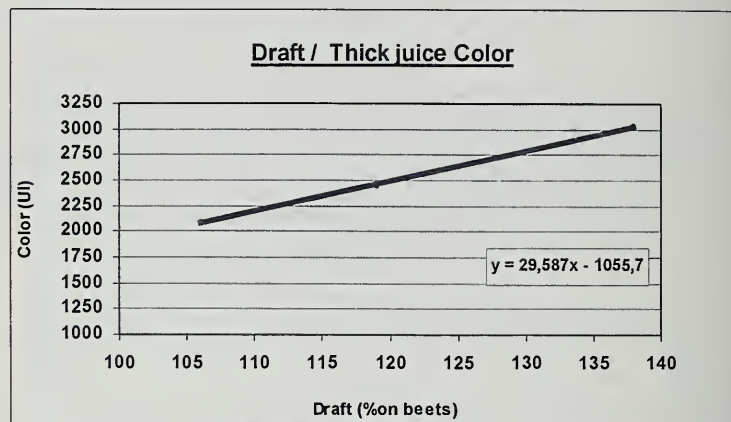
With southern beets of high beet quality there is a decrease in effective alkalinity at juice draft higher than 115%o.b. With low beet quality the effective alkalinity is much more lower (minus sign).

Figure 6



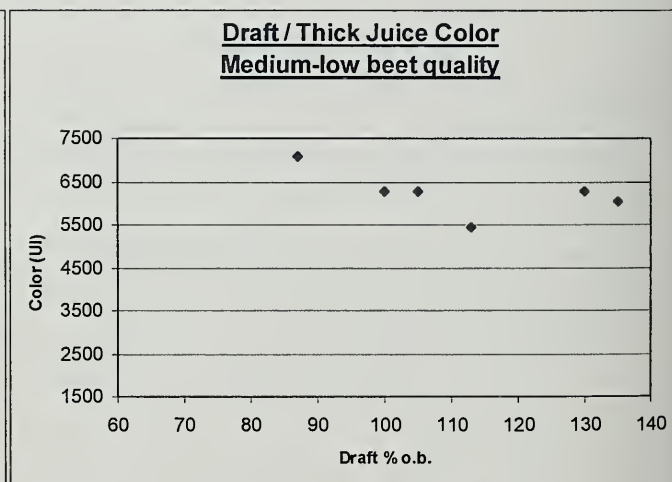
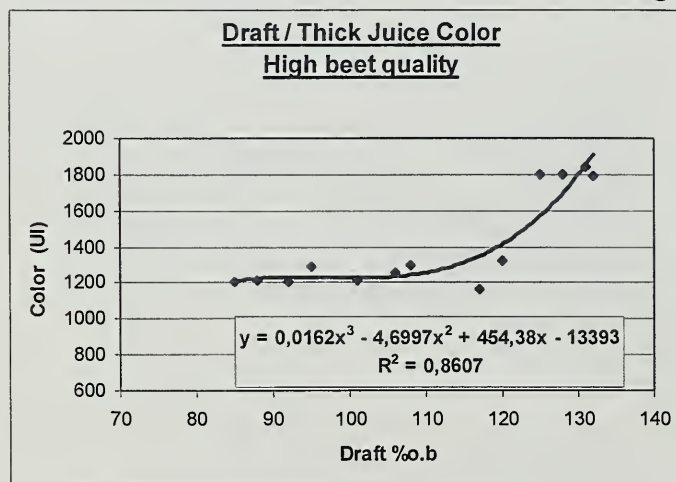
With northern beets, the color increase in the purified juice, when increasing draft from 105 to 125 was close to 25%. In thick juice the color increase was slightly higher (29%).

Figure 7



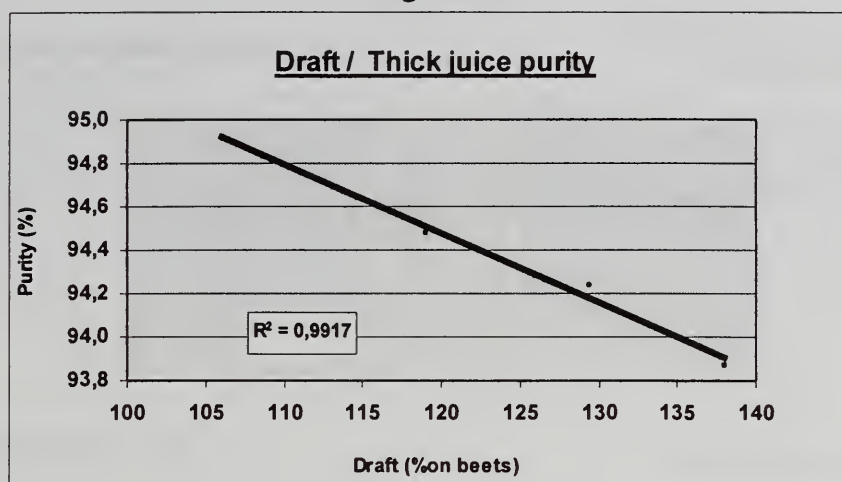
For southern beets of high beet quality, we found the optimal thick juice color at draft 110% o.b. For low beet quality the thick juice color was much higher:

Figure 8



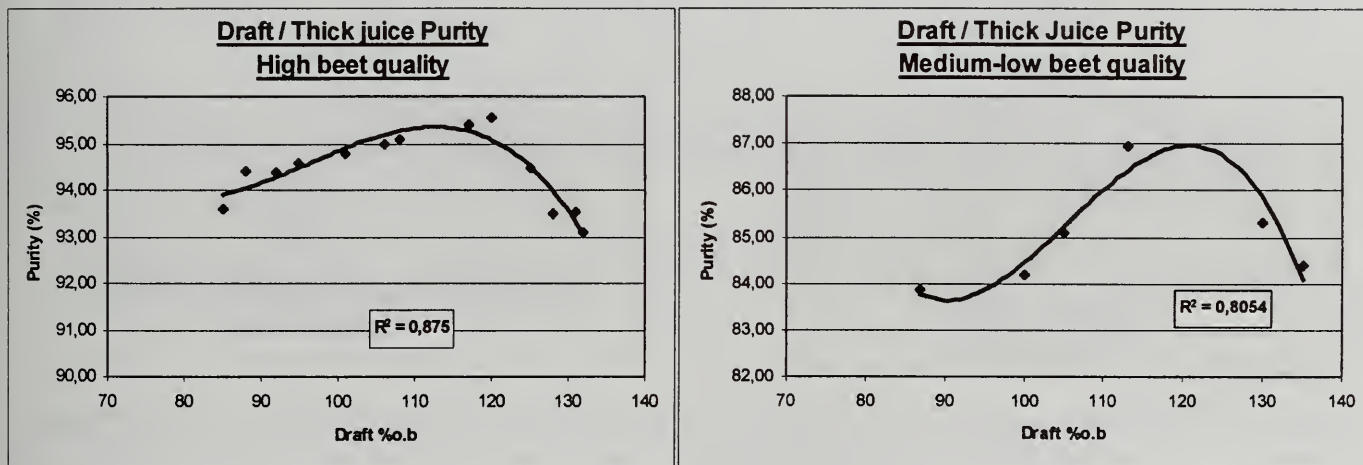
With northern beets, there was an increase of the non sugars entering the crystallization (25%) when increasing draft. That means lower thick juice purity at higher juice draft (Figure 9).

Figure 9



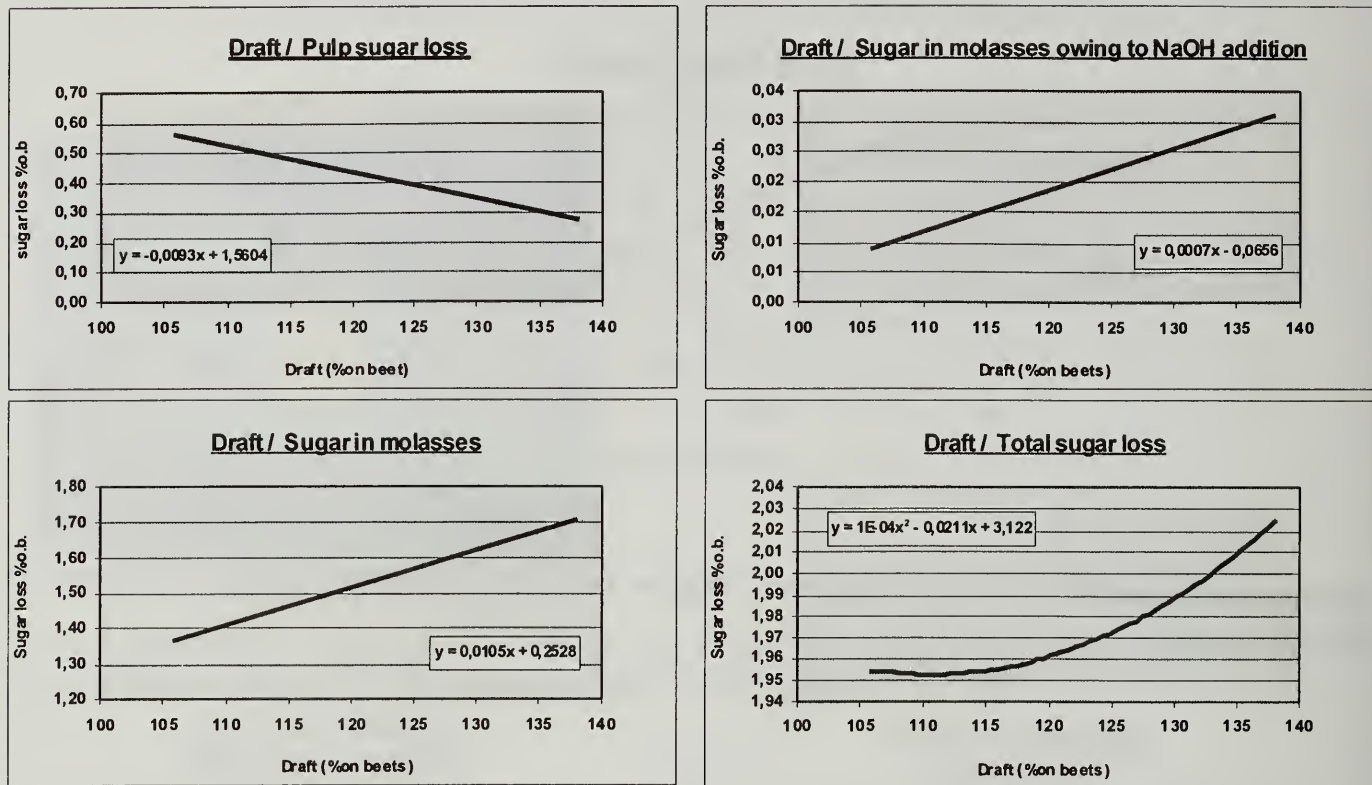
Southern beet results show that at juice draft higher than 110-115% there is also a decrease of thick juice purity.

Figure 10



We can see the overall Sugar losses for Northern beets versus juice draft in the next figures, increasing with juice draft higher than 115 %on beets (Figure 11):

Figure 11



For southern beets, we can show the sugar losses for high and low beet quality separately:

Figure 12

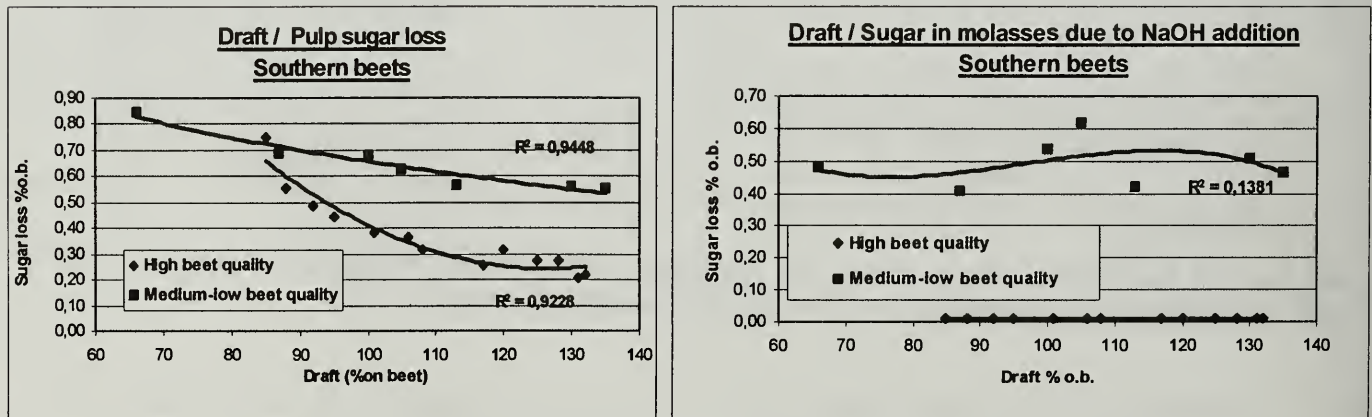
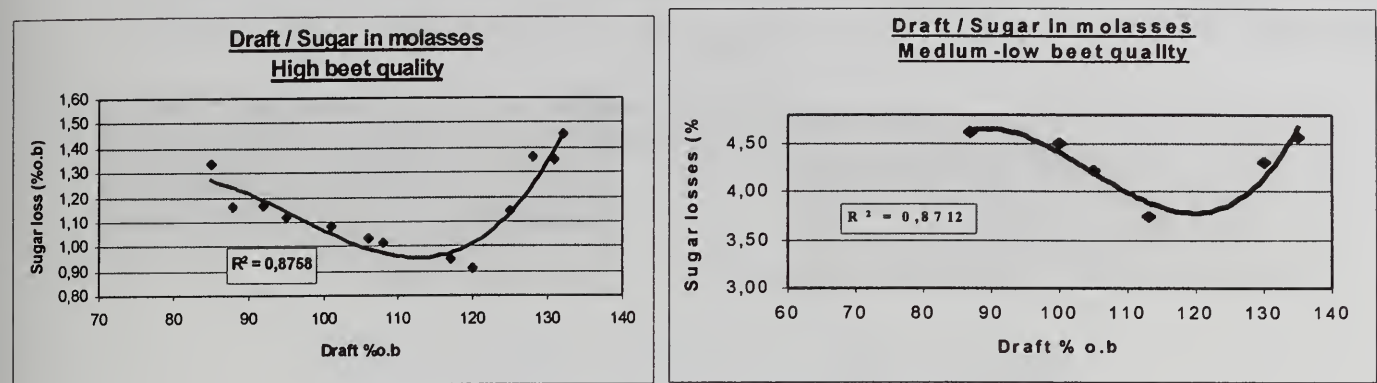
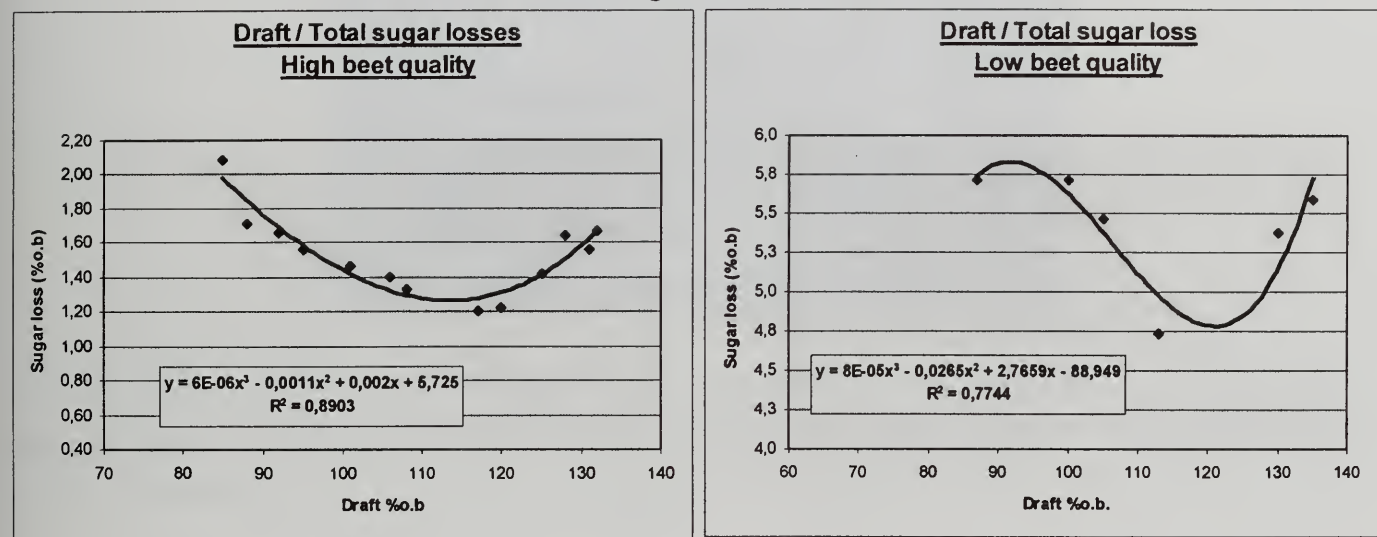


Figure 13



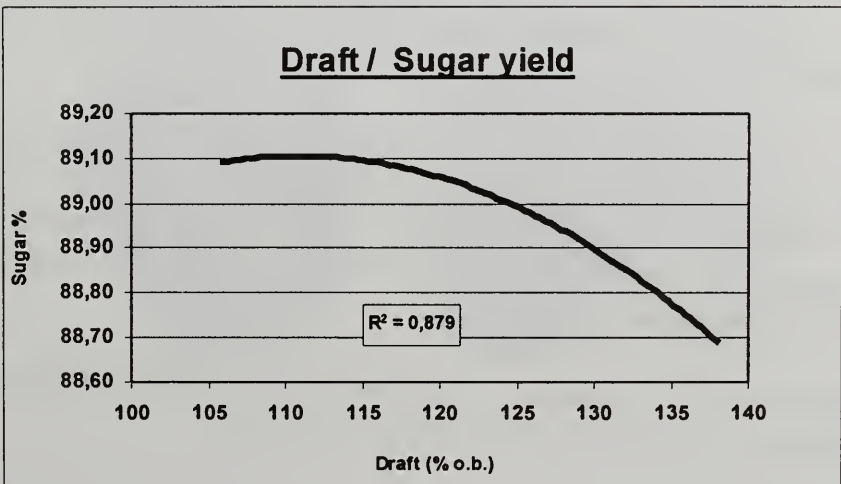
Overall sugar losses are shown in Figure 14.

Figure 14



There is a minimum of sugar losses at juice draft of 110-115% o.b. For northern beets, the sugar yield is constant from 105 to 115 %, decreasing with draft values higher than 115%.

Figure 15



For southern beets with high and low beet quality, the total sugar yield decreased at juice draft higher than 115%:

Figure 16

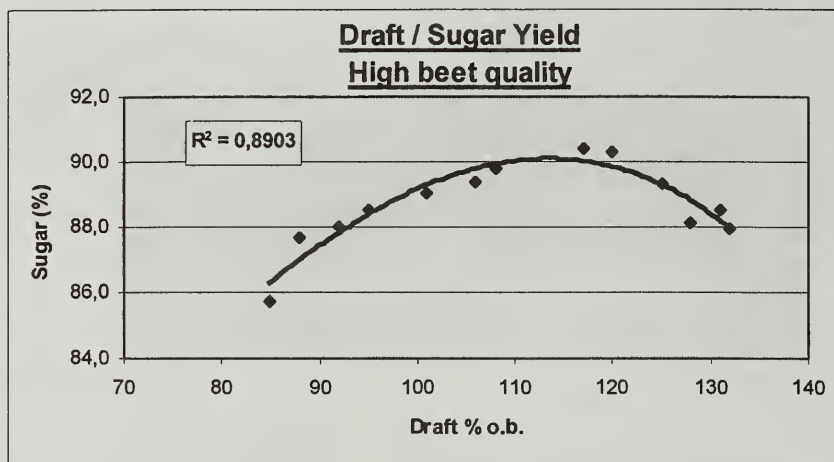
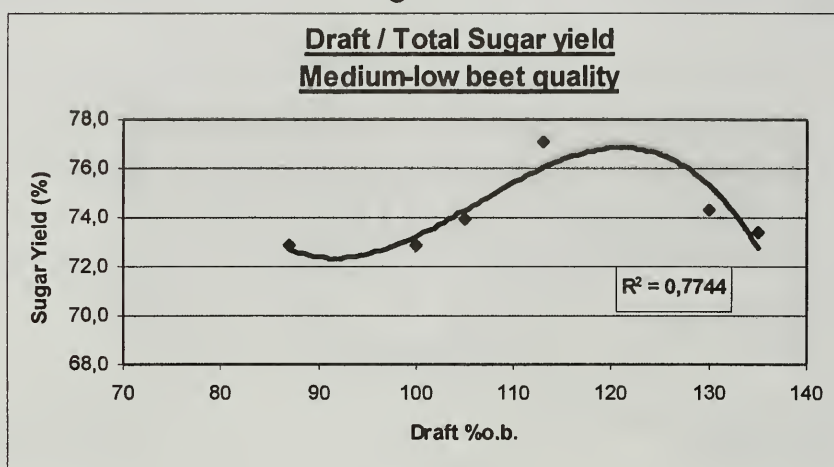


Figure 17



Oxygenation Degree

The target was to study the relationship between the O_2 concentration in the diffuser and the thick juice color formation.

During extraction in the presence of a high oxygen content, the polyphenols are oxidised to melanins, which are then removed in juice purification leading to a lower thick juice color. In our factories there are different types of extractors (RT, DDS and tower extraction). We know that oxidation of polyphenols to melanins may be different depending the extractor design and the oxygen content of the raw juices.

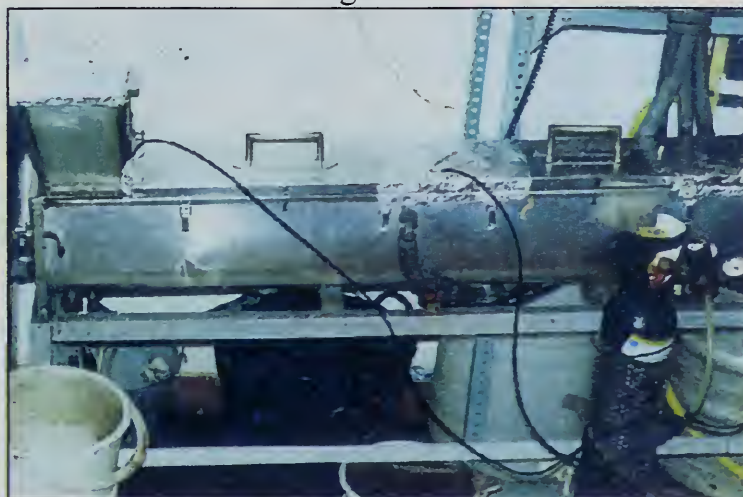
We studied the behavior of the diffusion process with three different degrees of oxygenation, to evaluate their influence on the quality of the juices and syrups.

- Process A: under normal conditions (reference).
- Process B: high anaerobiosis degree in diffuser (N₂ atmosphere).
- Process C: high aeration in diffuser (compressed air).

The operation methodology was the same for all the experiments.

- Draft: 120 % on beet
- Temperature: 72°C
- 4 points of gas addition, flow 4 l/min.

Figure 18



With process C (high O₂ content, left beaker in Figure 19) we obtained a raw juice with a blackish color due to melanin formation. In the absence of oxygen (Process B, high anaerobiosis degree) a grey color was noted in the diffusion juice, as well as a very clear pulps (see right beaker in Figure 19).

Figure 19



The color of the purified juice and thick juice in the anaerobic extraction was 22% and 27% higher respectively than in the aerobic process:

Figure 20

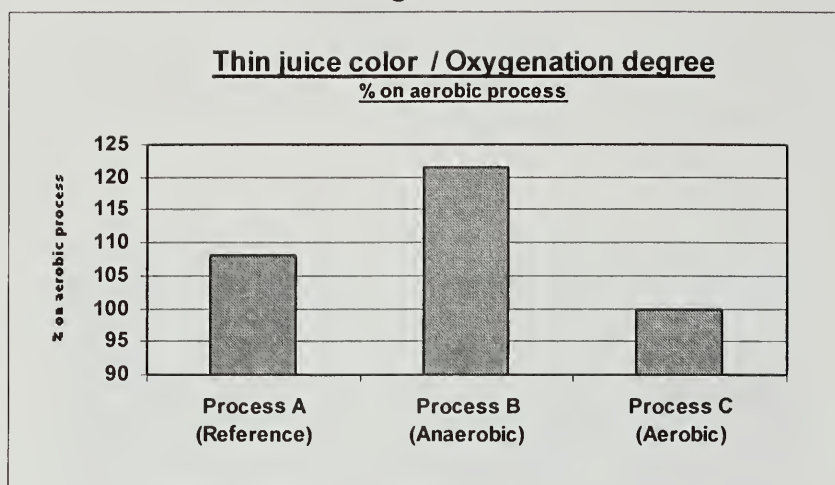
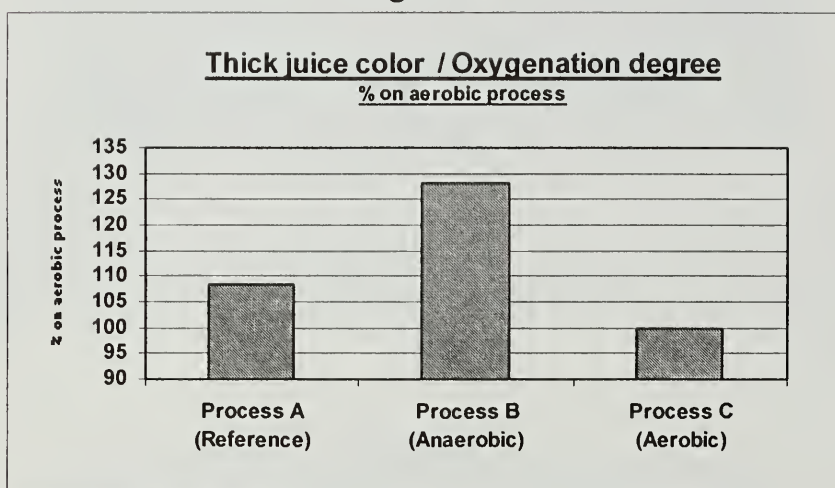
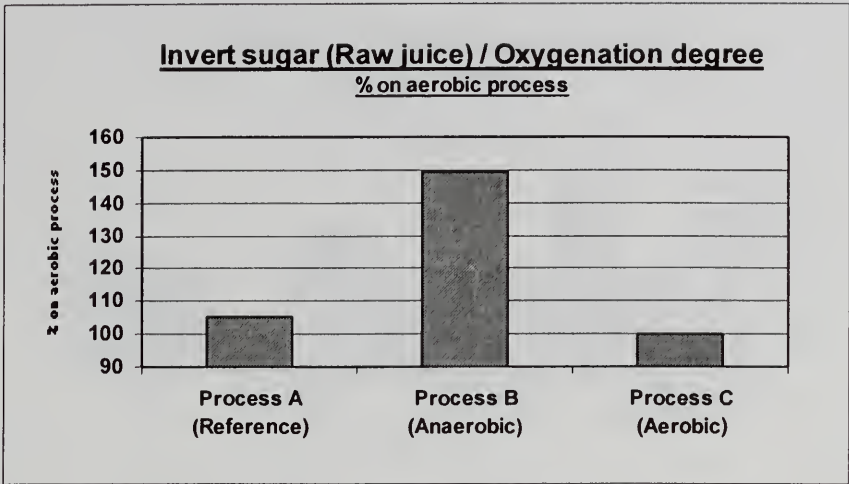


Figure 21



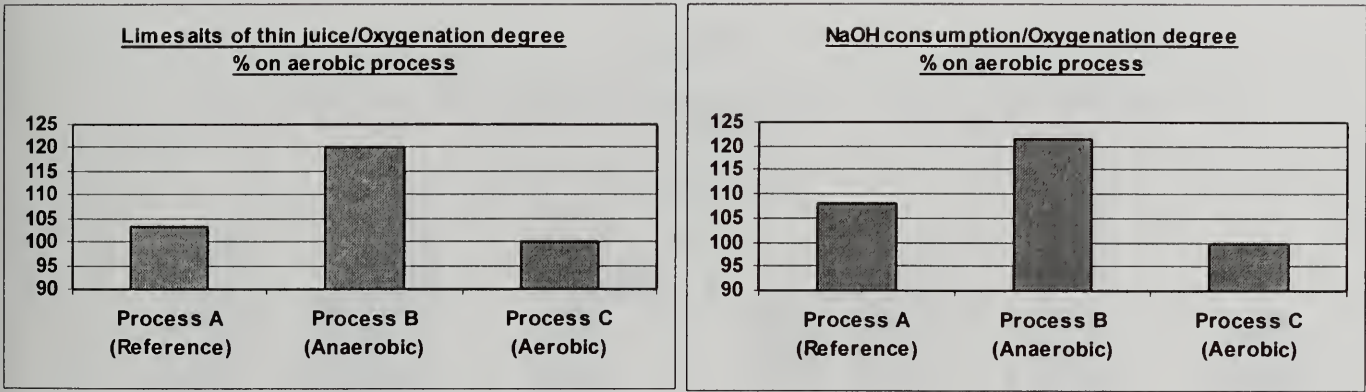
We found a higher content of reducing sugars in the anaerobic process. The oxygen content could influence the type of metabolites present in raw juice.

Figure 22



The aerobic process gave lower calcium salts and NaOH consumption (Figure 23).

Figure 23



Extraction Temperature

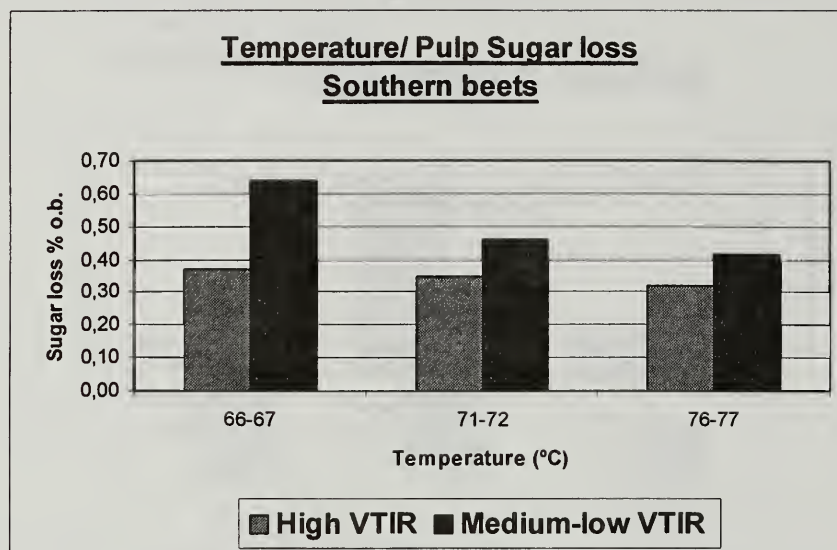
With northern beets we did a preliminary study with extreme temperatures (65°C and 80°C) in comparison to the normal processing temperature of 72 °C.

For southern beets we studied the sensitivity of this parameter on the process, obtaining different results for high and low beet quality:

- For high beet quality , temperature makes no difference between 66-77°C.
- For low beet quality the temperature is the most important parameter for extraction process optimization. There is a pronounced optimum at 71-72°C.

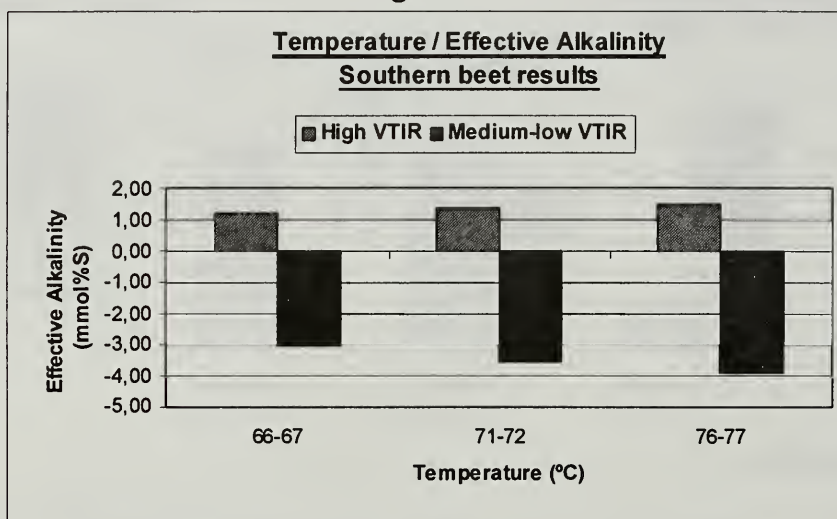
The overall results are shown in Figures 24, 25 and 26.

Figure 24



The pulp sugar losses decreased as temperature increased.

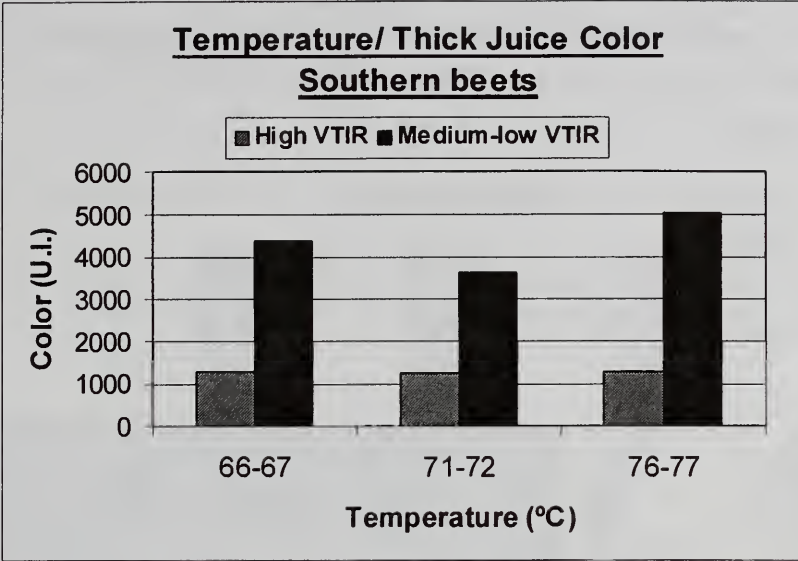
Figure 25



The effective alkalinity was more negative (minus sign) at higher temperatures with low technological beet value.

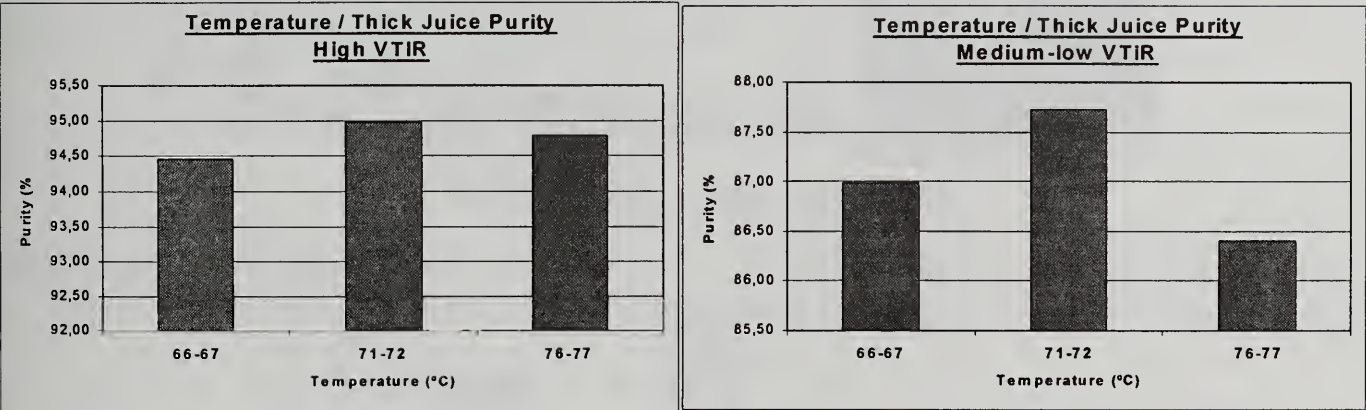
The best thick juice color was obtained at 71-72 °C, as shown in Figure 26.

Figure 26



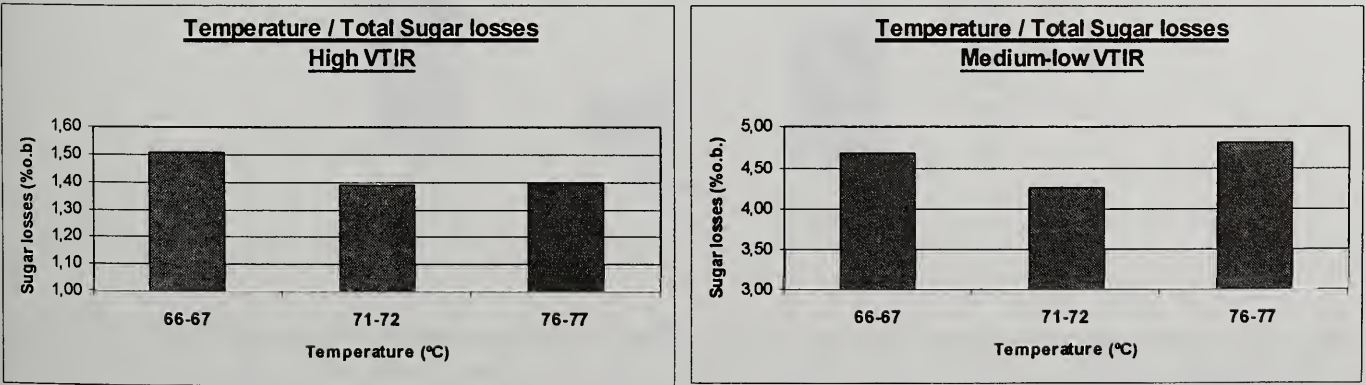
The optimum thick juice purity also occurred at 71-72 °C (Figure 27).

Figure 27



Regarding sugar losses, the minimum was at 71-72°C, and it was more pronounced for low beet quality (Figure 28).

Figure 28



Cossette Quality

We studied the behavior of the diffusion process with three qualities of cossettes, as well as the influence on the quality of the juices and syrups. The experiences carried out were done with three qualities of cossettes:

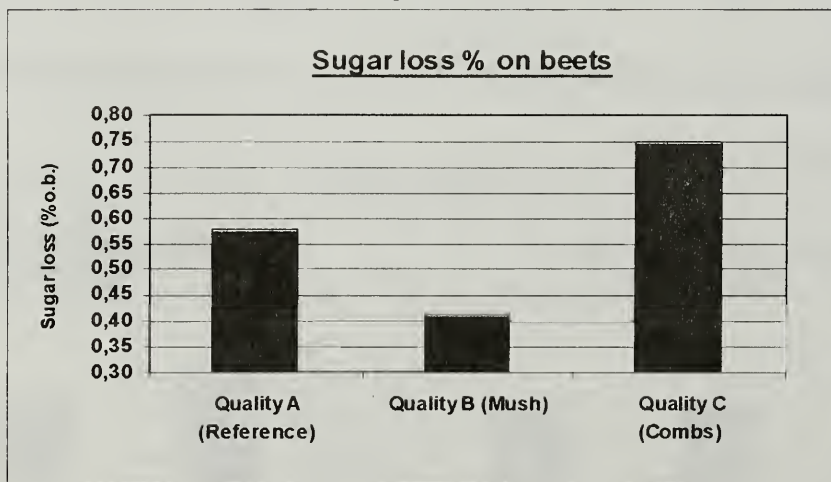
- Quality A of reference with a Silin number of 18, with a minimum mush content < 1% and without “combs”.
- Quality B with a high mush content, close to 50%.
- Quality C with a high percentage of “combs”, close to 50%.

Figure 29



An the increment in the quantity of “combs” in cossettes increased the sucrose losses in pulps. However, the relationship of non sugars/sugar increased with cossettes with high mush content.

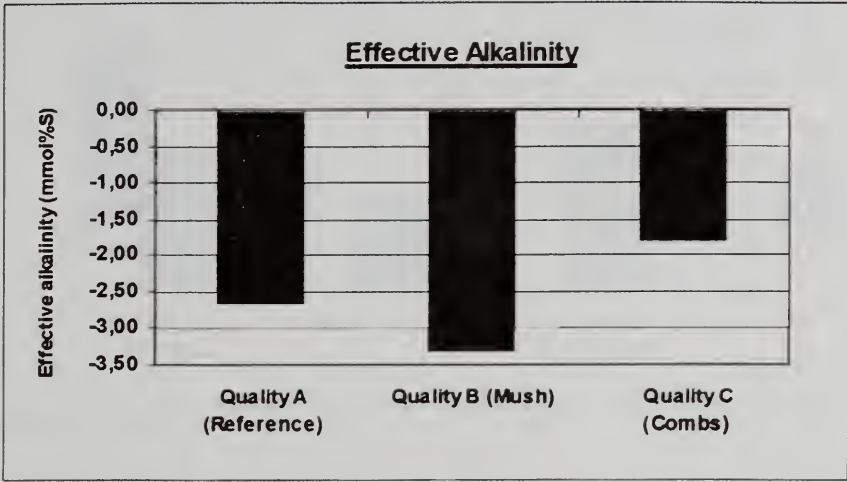
Figure 30



The concentration of reducing sugars in the diffusion juices coming from extraction processes with a higher mush content was notably higher than with good cossette quality. The need of

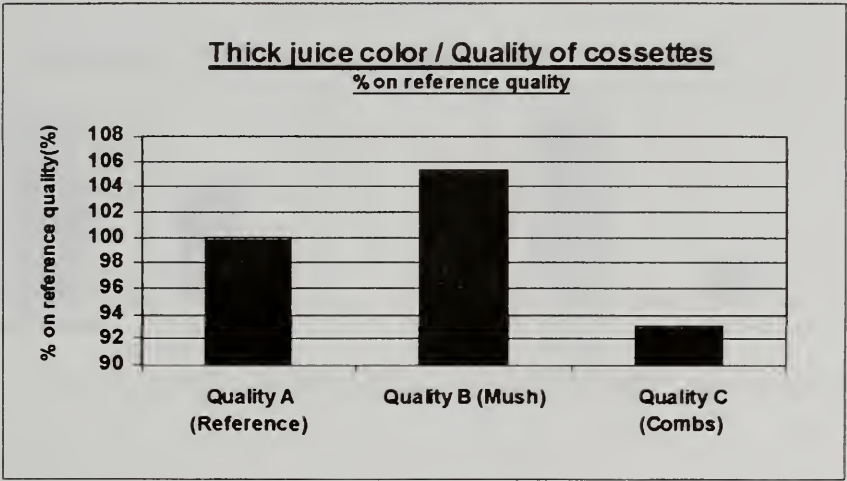
sodium hidroxide for juice softening was higher in the case of a massive presence of “mush”. With quality C, the effective alkalinity seemed to be higher than in the case of the reference, which translated iton a smaller consumption of NaOH in purification steps.

Figure 31



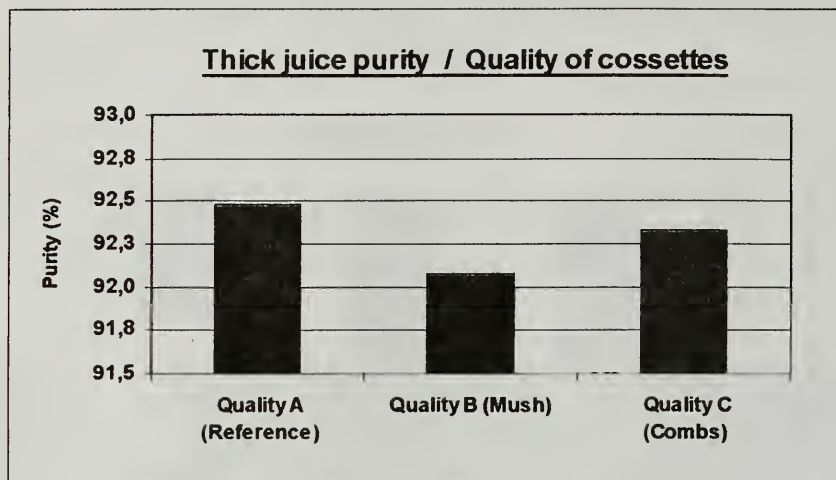
The color of thick juice was slightly higher in the presnece of mush. The higher presence of non sugars caused an increase in the color (Figure 32) .

Figure 32



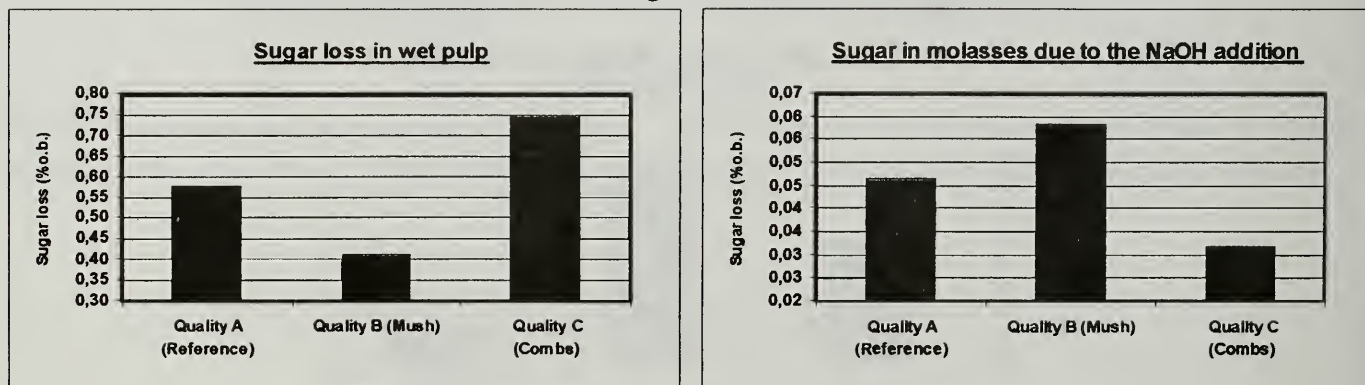
The quantity of non sugars in the thick juice was higher with cossetes with high mush.

Figure 33



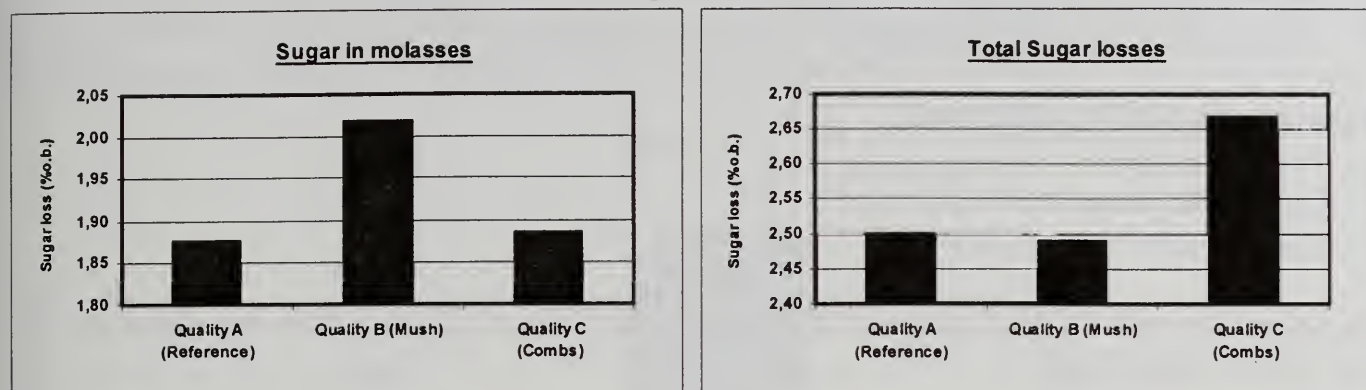
The presence of “combs” did not seem to affect to the purity of the thick juice. The highest sugar loss in diffuser was found with cossettes with a high quantity of combs. The biggest indirect losses (sugar loss in molasses) caused by the addition of NaOH in juice purification was found with cossettes with high mush.

Figure 34



The total losses were largest with Quality C, having a high percentage of “combs”.

Figure 35



Effect of Press Water Return

The effect of press water return was studied in order to investigate the expected sugar yield gain and also the negativ effects on thick juice quality. As referenced by van der Poel (1998), press water return should not be regarded from the viewpoint of a sugar yield gain but rather as a measure to achieve a closed extraction system without waste water discharge.

We did at laboratory scale the following process from press water to thick juice.

Press water from two factories in the south (autumn sown beets):

- Factory A: high beet quality
- Factory B: low beet quality

Preliminary concentration up to Bx 15 - 17.

Standard juice purification

Evapo-juice concentration

Determination of sugar yield

The data corresponded to 24 purifications conducted with the press water of each factory.

Press water Factory A

- Draft: 114 % on beet.
- Dry substance content: 3.25 %
- Press water flow: 45.6 % on beet.

Concentrated purified press water:

- Purity: 75.34 %
- Color: 6138 I.U.
- Calcium salts: 2.55 g CaO%Bx

Press water Factory B

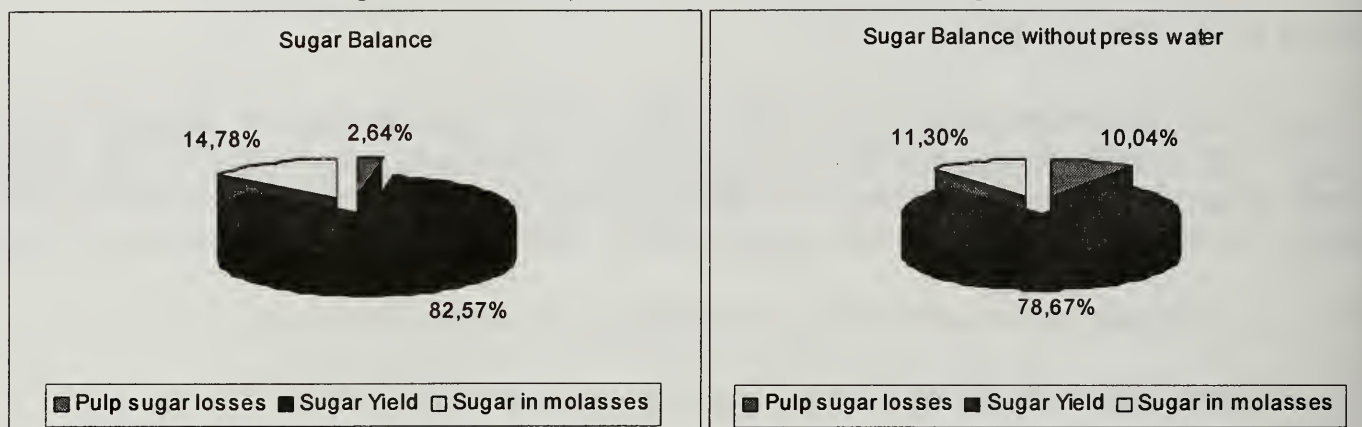
- Draft: 121 % on beet.
- Dry substance content: 2.7 %
- Press water flow: 48.4 % on beet.

Concentrated purified press water:

- Purity: 56.6 %
- Color: 6721 I.U.
- Calcium salts: 5.56 g CaO%Bx

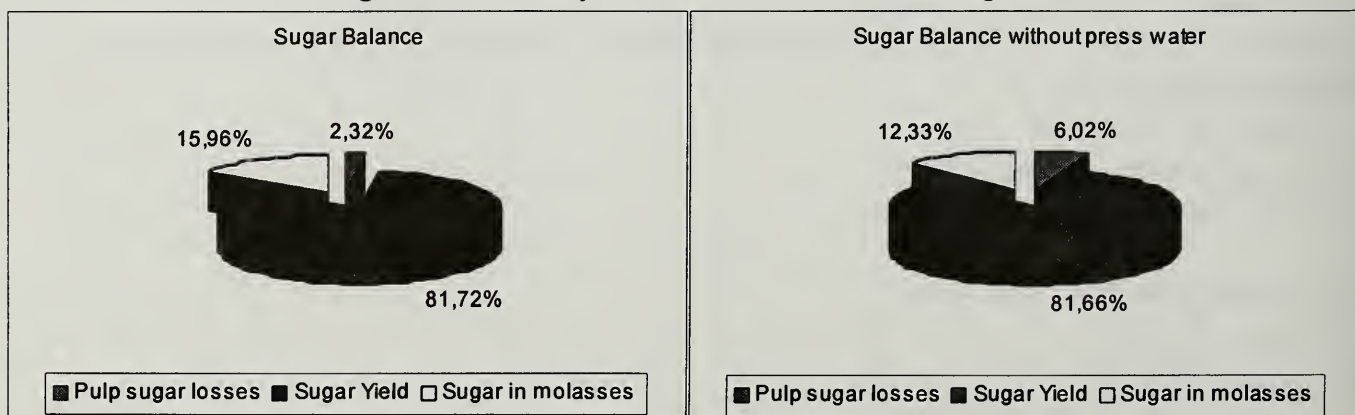
Applying formula IVRS (Instituut Voor Rationele Suikerproductie), we can calculate the recoverable white sugar.

Figure 36. Factory A. Mass balance % on Sugar.



With the same calculation we can evaluate the incidence of water press return in the sugar yield of factory B.

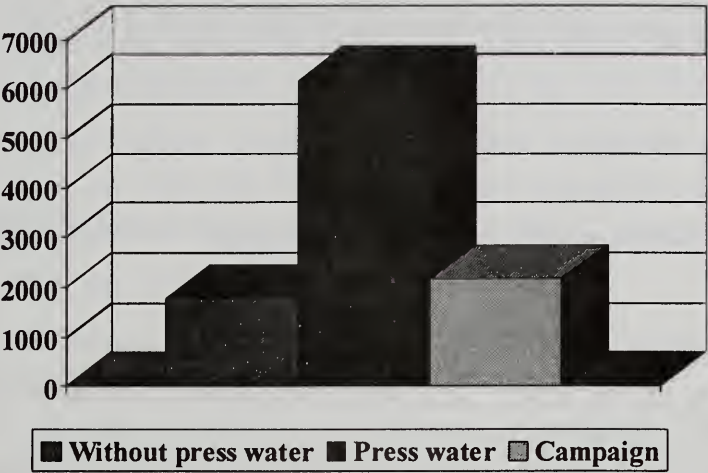
Figure 37. Factory B. Mass balance % on Sugar.



Influence on Thick Juice Color Formation

Color obtained in the campaign in juice before evaporation = 2,156 I.U.
Color obtained with the press water concentrated and purified = 6,144 I.U.
By balance we calculated the obtainable color without press water = 1,764 I.U.

Figure 38

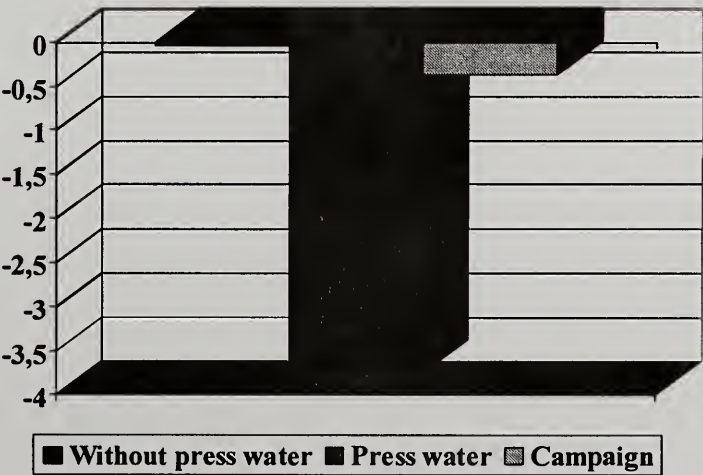


We can conclude that press water return caused a rise in color of 22 %.

In the same way, we calculated an increase of invert sugar content due to the press water return of 60%. This increment also produces an increase of color, already evaluated, but by another one when degrading itself in purification to acids it is going to cause an increase in the consumption of soda ash, with the negative repercussions that this has on the color and exhaustion of molasses.

Influence on Effective Alkalinity

Figure 39



The effective alkalinity increased NaOH consumption by 900 g/Tm beet, which means more sugar in molasses (0.4% o.b). We found a conflict between a benefit (recovered saccharose), and opposite effects caused by decreasing quality.

CONCLUSIONS AND SUMMARY

We have to separate good from low beet quality results, because of the important variability of harvested beets in Spain.

For good beet quality the results are well known:

- Temperature makes no difference between 66-77°C.
- Juice draft has an optimum at 105-110% o.b. with regard to sugar yield, thick juice color and effective alkalinity.

For low beet quality results:

- Temperature is the most important parameter for extraction process optimization. There is a pronounced optimum at 71-72°C.
- Juice draft is a secondary variable, influencing sugar yield, and is optimum at 110-115% o.b.
- Water press return: No sugar yield increase or decrease was found with low quality press water. All the sugar returned to extraction process from the press water will end up in molasses.
- Press water return causes a deterioration in thin juice quality:
 - Color increase of 22%.
 - Effective alkalinity decrease of 88% (soda ash consumption increases from 103 to 900 g/Tm beets).

Results confirm that cossette quality and oxygenation degree have a great influence in the extraction process.

- Higher aeration in the extraction process provokes a lower thick juice color (25%).
- High cossette quality reduces 35 % pulp sugar losses.

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OPTIMIZATION OF BEET SUGAR PRODUCTION BY CAMPAIGN DURATION: EVOLUTION OF THE INDUSTRIAL-TECHNOLOGICAL BEET VALUE OVER 200 DAYS IN NORTHERN SPAIN

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ABSTRACT

As more benchmarking is done in w-rld wide sugar production, the more important becomes the annual exploitation time in order to reduce the fixed costs and the financial capital expenses. The campaign duration is therefore becoming one of the most important competitiveness criteria.

As sugar beet is a living vegetable raw material, we are obliged to consider the relatively short shelflife of the beets fixing the adequate campaign beginning and duration. Different ways to store the harvested beets at cold temperature, above and below the freezing point, are especially well known in the US. The climatic conditions oblige harvest of the beets before the outside temperature drops.

The question is what will happen when the weather allows us to leave the beets in the ground. In this case, the plant growing cycle is not interrupted.

This present R&D project is based on about 60,000 beet and juice samples, which were analyzed for industrial economical criteria. The project target was to check the feasibility of a new harvesting logistic, as the northern Spanish sugar beet area (80% of the total national sugar production) presents very special climatic conditions which could potentially allow to leave the beets into the ground for more than 180 days.

Conclusions are presented, as well as new project activities suggested.

INTRODUCTION AND TARGET

The principle aim of the nd R&D project could be described as follows:

An annual exploitation time maximization results in:

- Increase of the industrial facility productivity
- Fixed cost reduction
- CAPEX reduction and concentration of the sugar production

One of the main features of the sugar harvest is the seasonal annual industrial exploitation time. This means an important limiting factor and disadvantage, as well as a very important potential opportunity. The continuously advanced optimization tendency of the main part of the sugar industry has resulted in important improvements of the agricultural and industrial transformation yields. The proportional impact of the fixed costs related to investments and campaign duration is becoming more important.

It could be stated that the agroindustrial optimization describes the following framework:

- Sugar production assurance and/or increase
- Agricultural production costs
- Industrial transformation costs
- Capital expenditure and capital costs
- Other fixed costs
- Ecoefficiency

In general, there is a trend to lengthen the beet slicing period, as observed in Europe and the United States. As this trend is mainly achieved through additional beet storage, the results are -- besides the above described advantages -- additional direct and indirect sugar losses and, therefore, additional production costs. Whether this measure is profitable depends mainly on the balance between additional savings and additional costs.

In northern Spain, where 80% of the total national sugar production is located, there could exist another option:

The target of this R&D project was to analyse if the climatic conditions allows us to leave the beets over a long time in the ground, avoiding any intermediate storage and therefore industrially relevant changes in beet composition. As the growing cycle is not interrupted, the question is if this Spanish option could be one step towards modulating the vegetation period according to an optimal agro-industrial balance.

Trial Period:

- Three periods:
 - 2000-2001 Campaign .
 - Start: 9 October 2000
 - Final: 28 April 2001
 - Total: 200 days
 - Three zones
 - 1997-1998 Campaign. 166 days, two zones
 - Start: 1 October 1997
 - Final: 16 March 1998
 - Total: 170 days
 - Three zones
 - 1996-1997 Campaign. two zones
 - Start: 8 October 1996
 - Final: 1 April 1997
 - Total: 170 days
 - Two zones
- 60,000 total beet and juice samples were analyzed

**INDUSTRIAL-TECHNOLOGICAL BEET VALUE IN THE NORTHERN SPAIN:
NORTHERN VTIR EQUATIONS (SPRING BEETS SOWING)**

2000/2001 Campaign

Results:

- Slight drop in effective alkalinity (Figure 1).
- Slight increase of thick juice colour (Figure 2).
- Thick juice purity keeps constant from first October until the end of May (Figure 3).
- During this period the sugar yield did not decrease in significant figures (Figure 4).

Figure 1

2000-2001 Campaign
Effective Alkalinity (VTIR 2002)

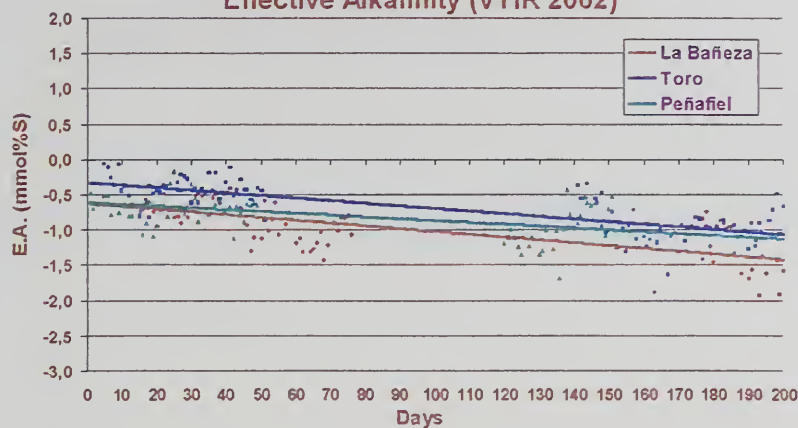


Figure 2

2000-2001 Campaign
Thick juice Colour (VTIR 2002)

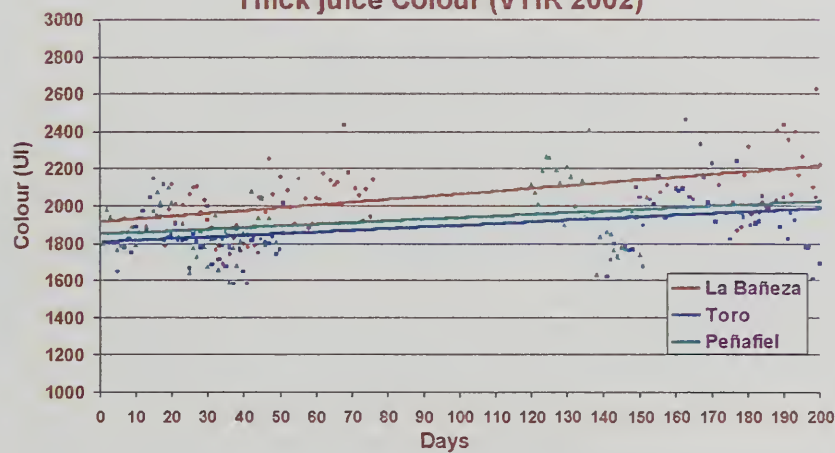


Figure 3

2000-2001 Campaign
Thick Juice Purity (VTIR 2002)

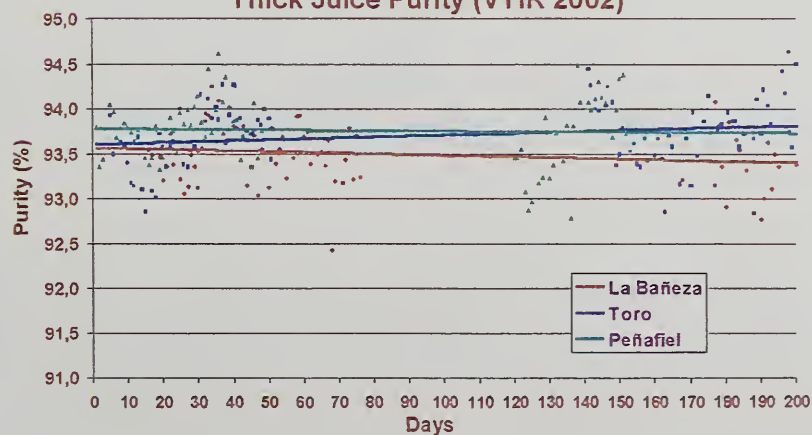
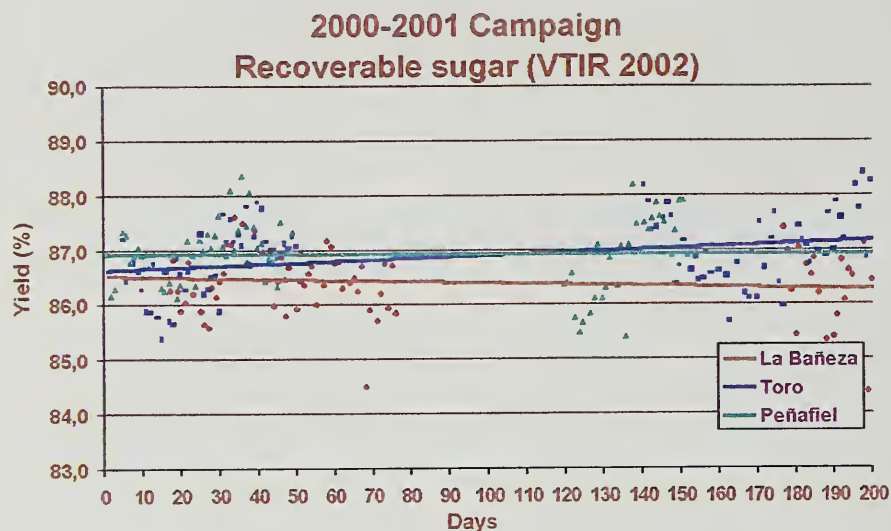


Figure 4



1997/1998 Campaign

Results:

- VTIR was stable during 170 days as we can be sees from the results for effective alkalinity (Figure 5), thick juice color (Figure 6) and thick juice purity (Figure 7).
- Industrial sugar yield was constant during the whole period (Figure 8).

Figure 5

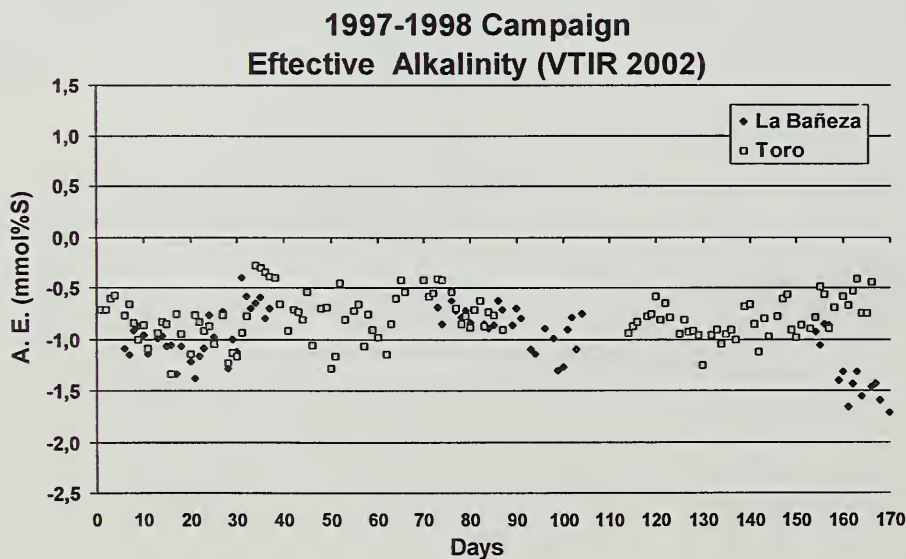


Figure 6

**1997-1998 Campaign
Thick Juice Colour (VTIR 2002)**

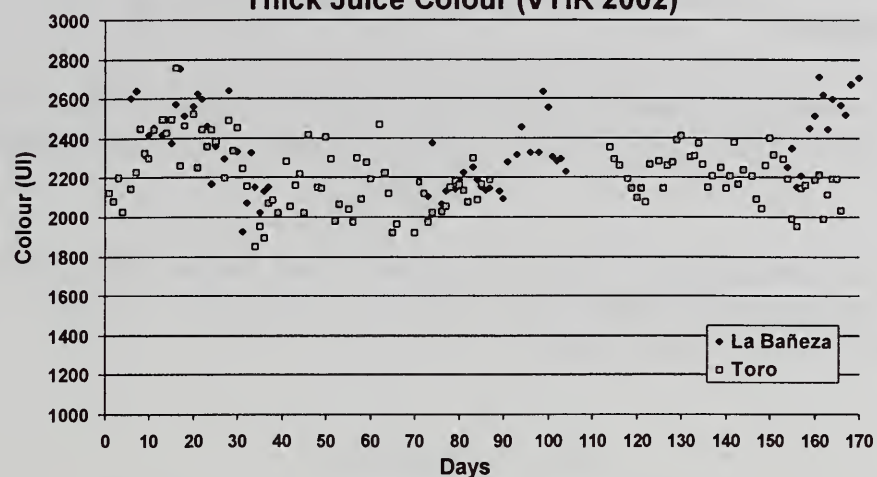


Figure 7

**1997-1998 Campaign
Thick Juice Purity (VTIR 2002)**

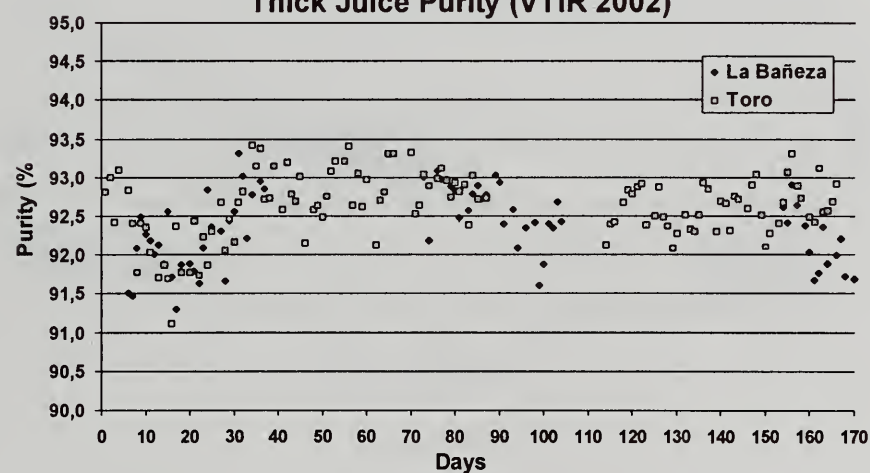
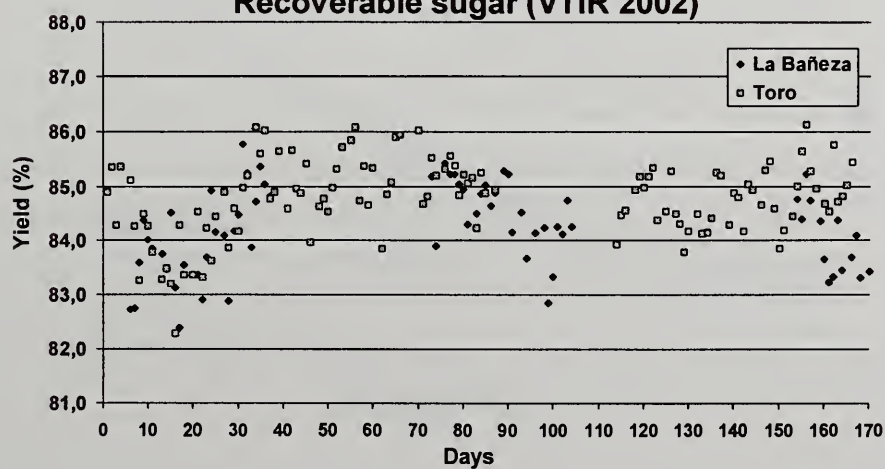


Figure 8

**1997-1998 Campaign
Recoverable sugar (VTIR 2002)**



VTIR 2002 VERSUS DIFFERENT OTHER EUROPEAN ECQATIONS

2000/2001 Campaign

- Excellent correlation between VTIR 2002 equation and Devillers 1988 (Figure 9) and Pollach 1991 (figure 10) equations (both of them take into account reducing sugar content).
- Low correlation between VTIR 2002 equation and Burba 1993 (Figure 11), Buchholz 1995 (Figure 12) , Huijbregts 1999 (Figure 13) equations (the last two equations don't take into account reducing sugars).

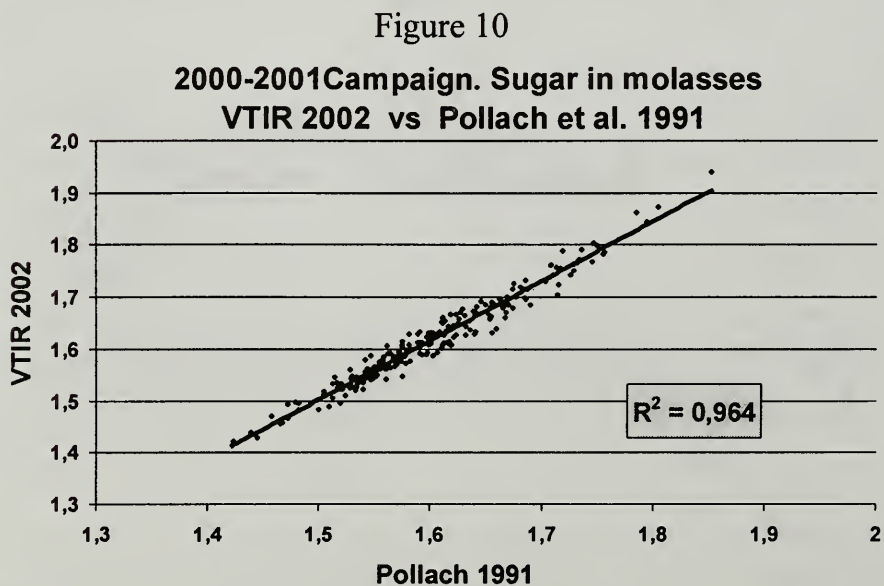
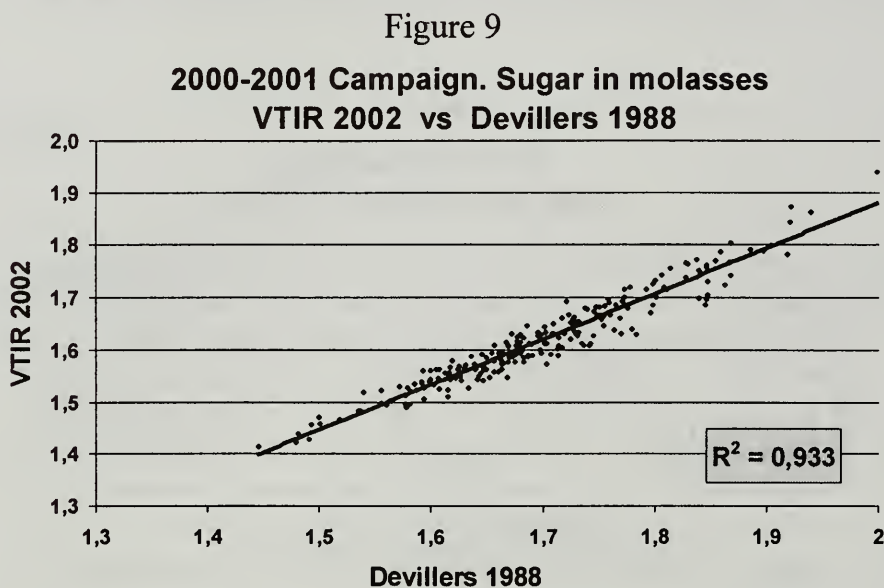


Figure 11

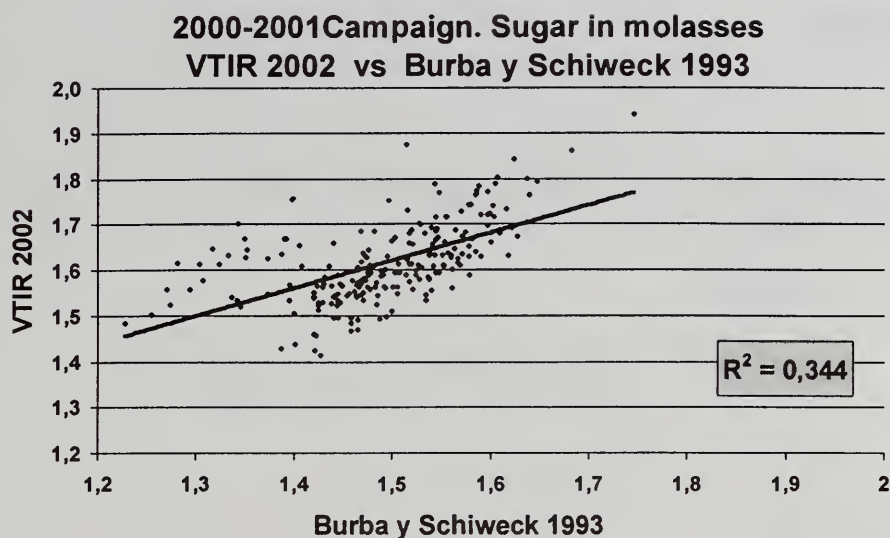


Figure 12

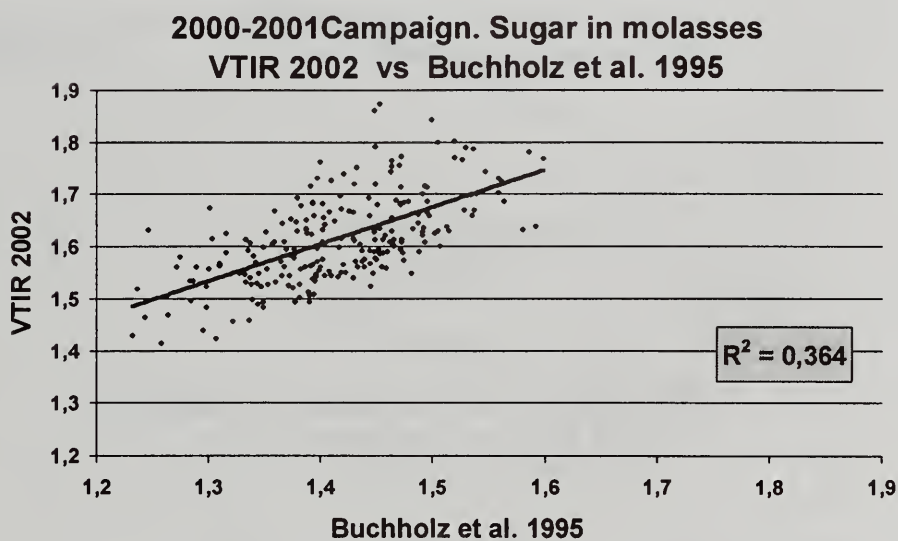
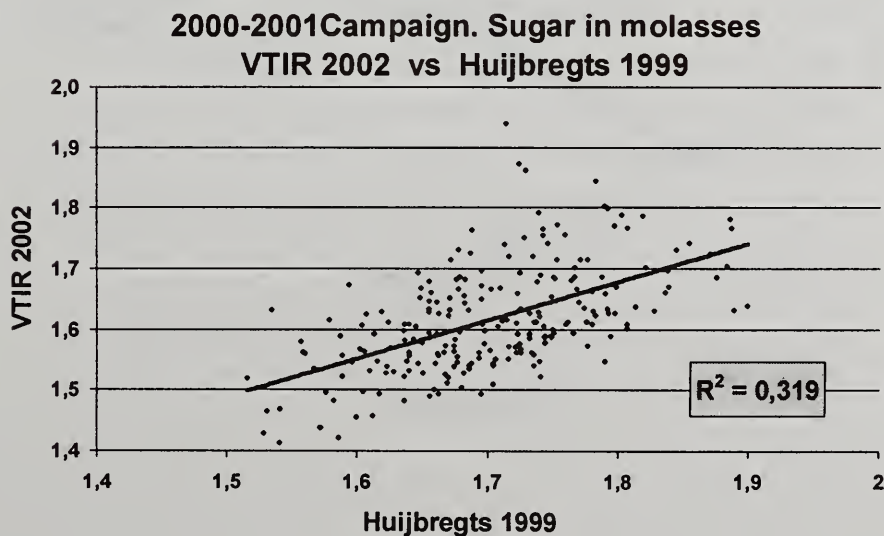


Figure 13



1997/1998 Campaign

Results:

- Good correlation between VTIR 2002 equation and Devillers 1988 (Figure 14) and Pollach 1991 (Figure 15) equations.

Figure 14

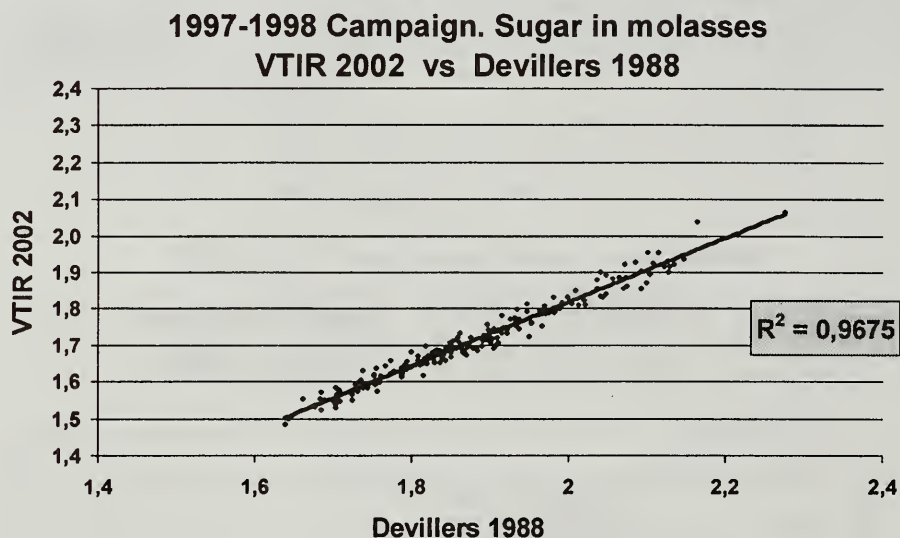


Figure 15

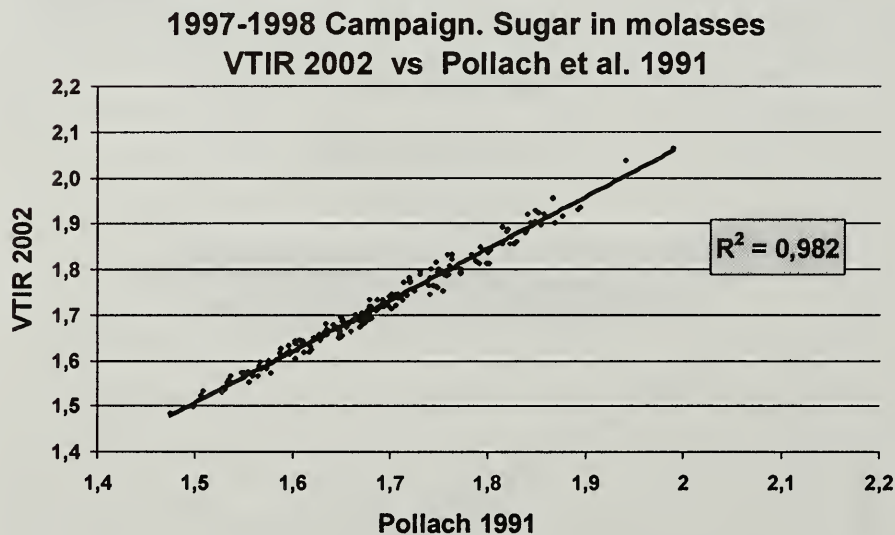


Figure 16

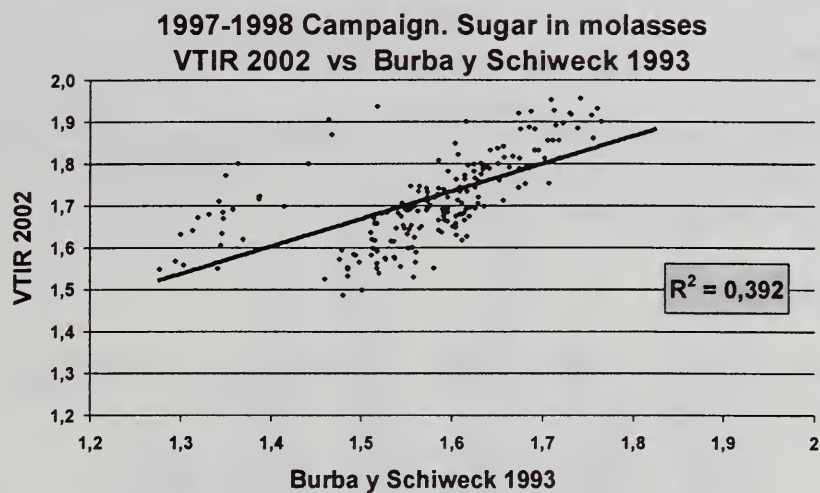


Figure 17

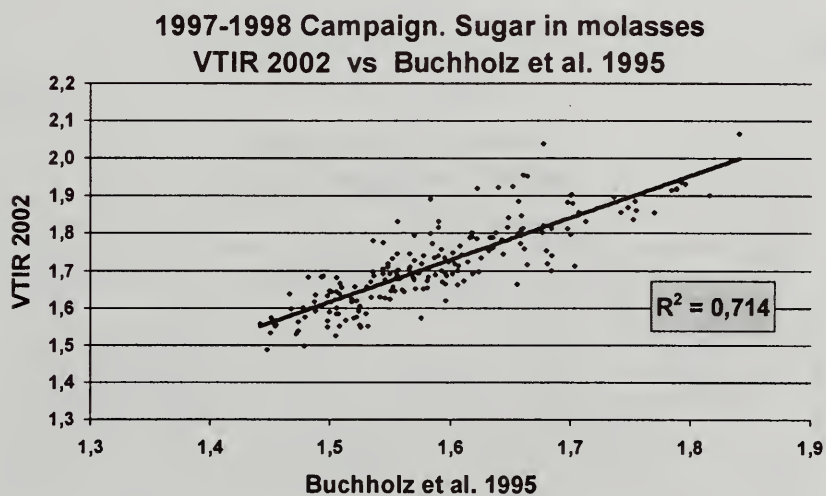
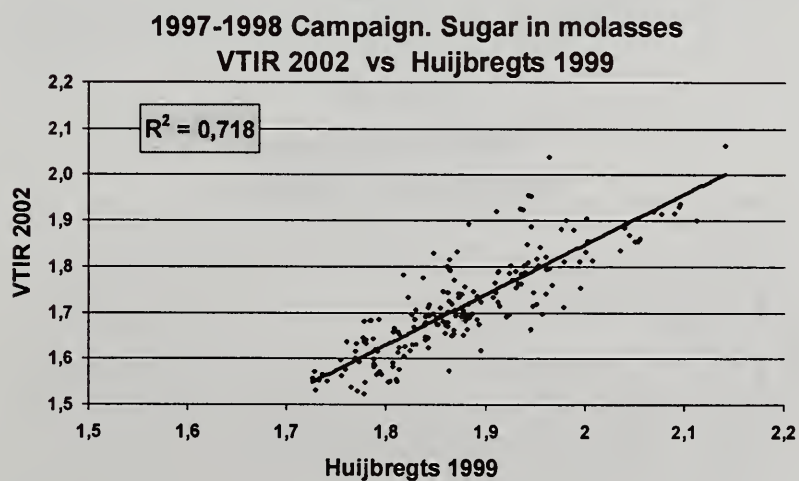


Figure 18



COMPARISON AND VERIFICATION WITH FACTORY ANALYSIS

2000/2001 campaign

- The sugar factory results corroborated the VTIR predictions. Low increase in thick juice color and no significant differences in the purity of thick juice.
- Sugar quality remained constant for 200 days.

1997/1998 Campaign

- The sugar factory results corroborated the VTIR predictions. No significant differences in the purity and color of thick juice.

TEMPERATURES AND RAINFALLS

2000/2001 campaign

Figure 19

**2000-2001 Campaign
Minimum temperatures**

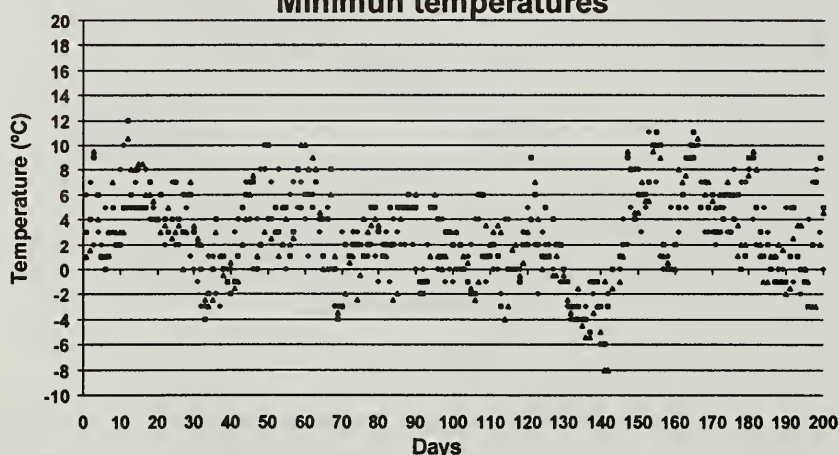


Figure 20

**2000-2001 Campaign
Maximum Temperatures**

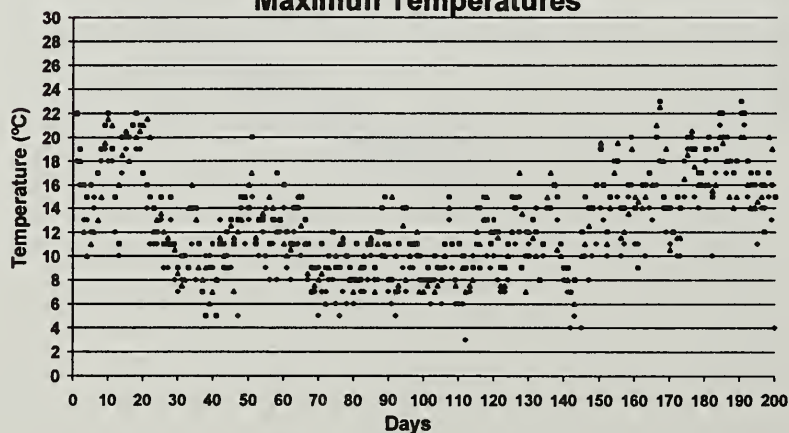
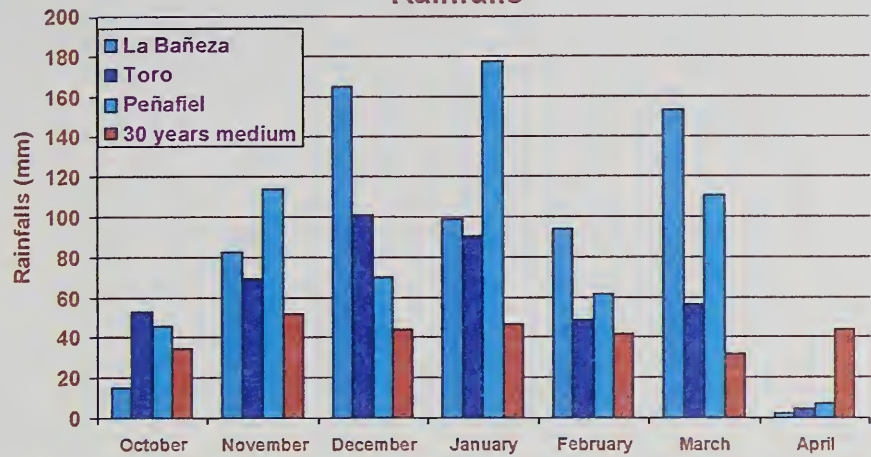


Figure 21
2000-2001 Campaign
Rainfalls



1997/1998 Campaign

Figure 22
1997-1998 campaign
Minimun Temperatures

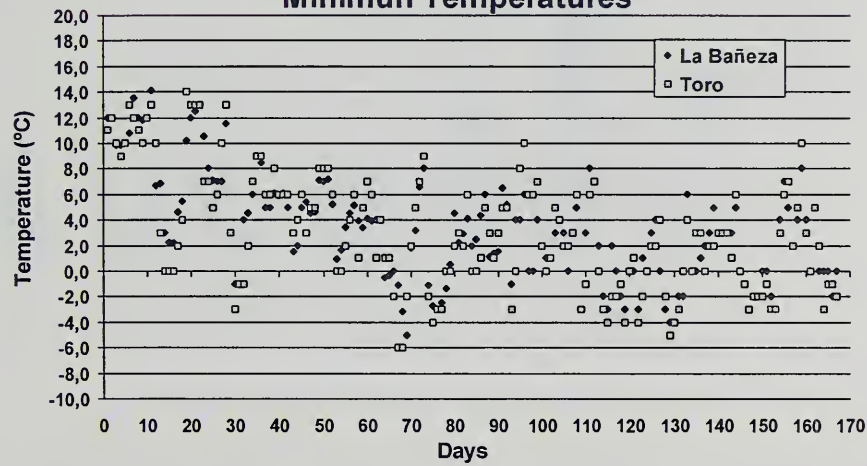


Figure 23

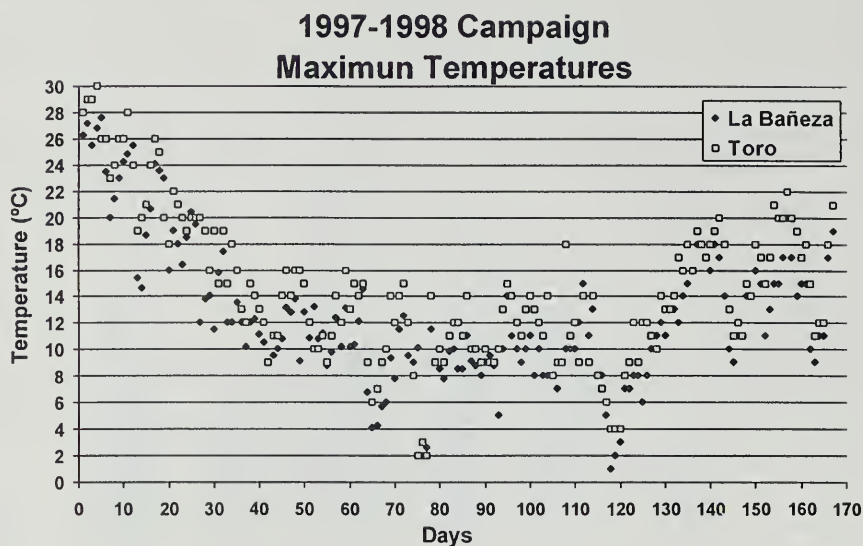
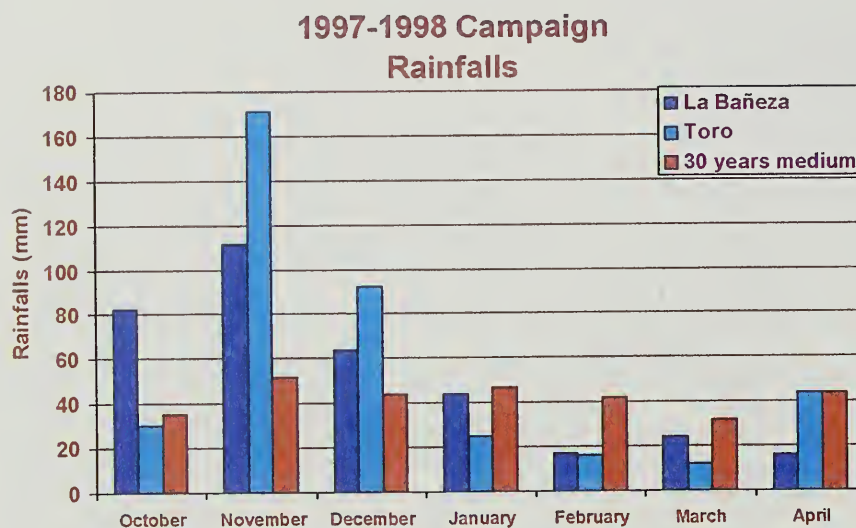


Figure 24



OTHER ASPECTS

- Affected hectares of late harvest: 11,000 Hectares (March and April)
- Other crops that can follow late harvest and can be planted even late April: corn, sunflower, cereal, fallow land.
- Disadvantages of late harvest:

Farmers do not have enough time to prepare land for the next crop.

Risk of frost -- beet deterioration.

Risk of rain -- campaign gets longer.

EVALUATION OF FROST RISK

- Historical data 20 last years (1980-2000)
- October - April
- North of Spain.
- Probability plots (Figures 25 to 31)

Figure 25

Normal Probability Plot for october 1980-2000

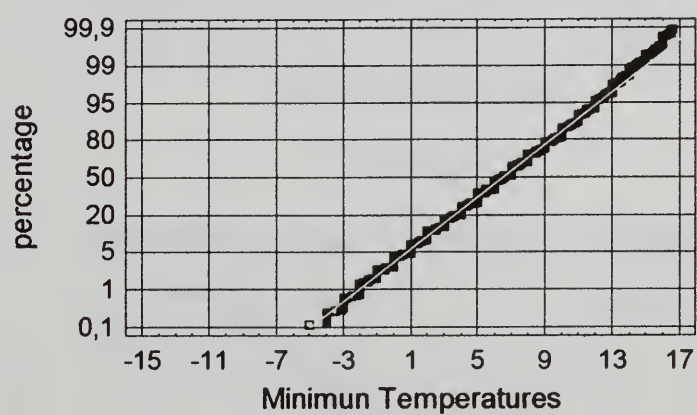


Figure 26

Normal Probability Plot for November 1980-2000

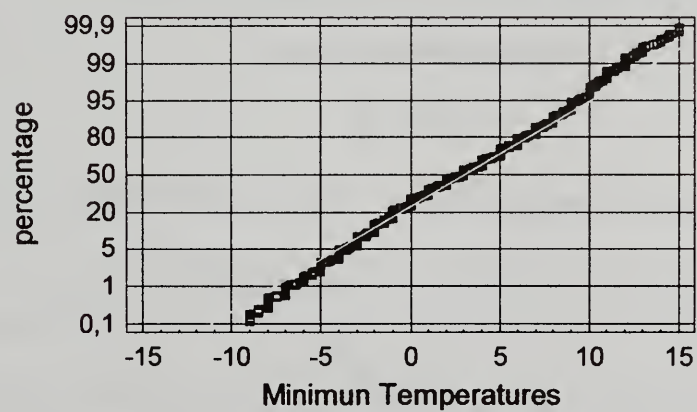


Figure 27

Normal Probability Plot for December 1980-2000

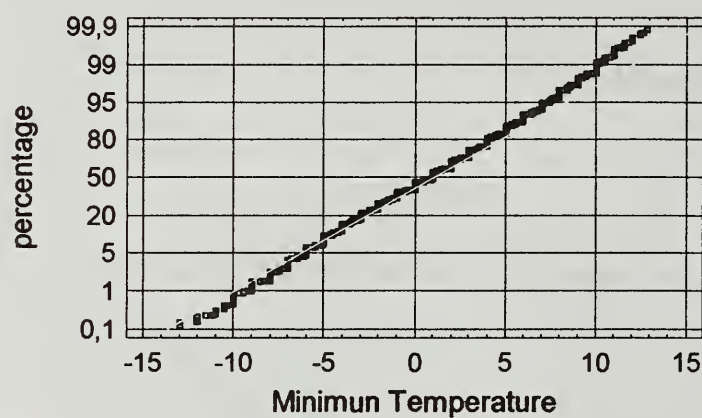


Figure 28

Normal Probability Plot for January 1980-2000

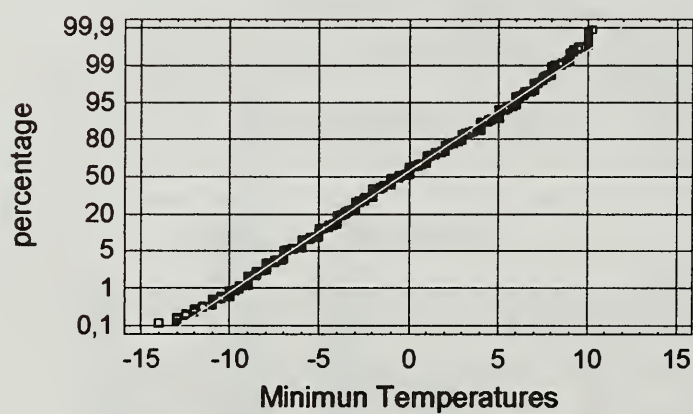


Figure 29

Normal Probability Plot for February 1980-2000

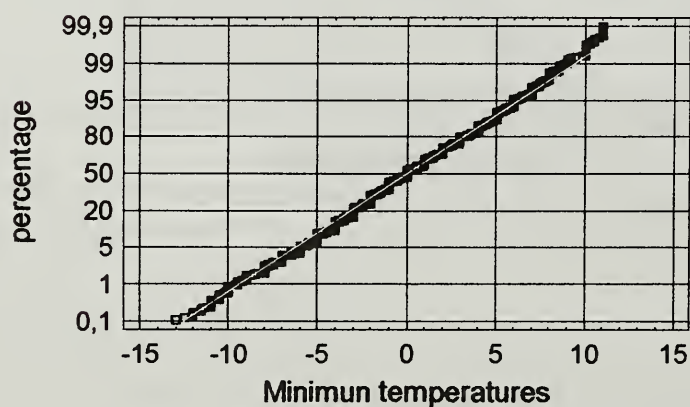


Figure 30

Normal Probability Plot for March 1980-2000

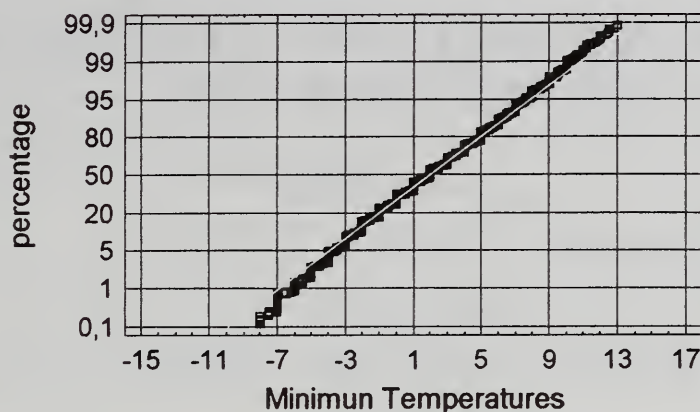
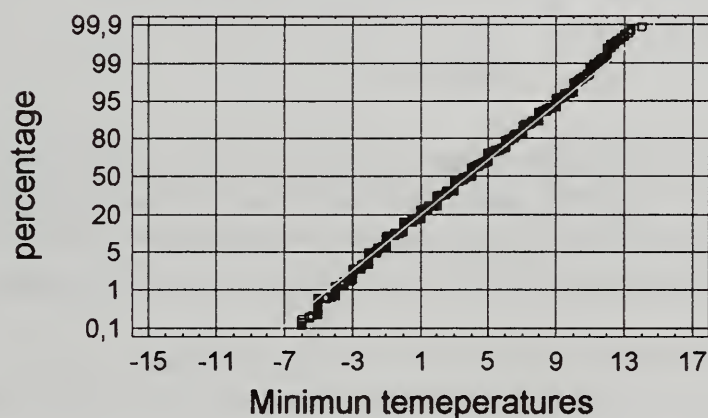


Figure 31

Normal Probability Plot for April 1980-2000



PROJECT CONCLUSIONS

- A newly developed method is applied to predict the technological-industrial value of northern Spanish beet (nonsugars, color and effectivy alkalinity).
- We can predict, besides the sugar yield, the color formation and effective alkalinity, as these three results are of economical importance for the industrial exercise
- The VTIR of the beets left in the ground over 200 days does not change.
- According to these results, the industrial explotation cost could be maintained over 200 days without any changes within the factory.
- There are different disadvantages to be solved: frost risk, following crops logistic, rainfalls influence.

WHITE SUGAR FROM CANE AT THE FACTORY THE IMPACT OF WSM

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Calgon Carbon Corporation, Pittsburgh USA

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Tongaat-Hulett Sugar Limited, Durban RSA

Wolfgang Fechter

South African BioProducts Limited, Durban RSA

ABSTRACT

A process to produce both direct refined sugar as well as a fermentable sugar feedstock from sugar cane has been developed by Tongaat-Hulett Sugar and AECI Bioproducts. The process designated the White Sugar Mill (WSM) process was introduced by Fechter et alia at ISSCT 2001. The process purifies partially evaporated juice employing ultra-filtration and ion exchange operations.

This publication re-summarizes the concept of the process and then deals with the aspects of integrating these new unit operations into an existing cane mill. Different operating scenarios are discussed to show the versatility of the process configuration.

The steam and energy balances illustrate how easily a mill can adapt to the new electrical and steam loads. Examples are provided for different pan-house and evaporator station arrangements.

The UF and resin processes generate new effluents and by-products. The properties and potential value of these streams are discussed. Finding useful homes for effluents and dealing effectively with the energy requirements of the WSM process promises to make it the future choice for sugar companies in a world where white sugar product demand continues to grow.

INTRODUCTION

In a recent publication at ISSCT 2001 Fechter, *et al.*, (1) introduced a new process referred to as the WSM (White Sugar Mill) process. WSM purifies cane juice at low brix before it becomes syrup feed to the factory vacuum pans. WSM pan products are white refined sugar and a high-grade molasses. Impurities extracted from the juice become by-products suitable as liquid fertilizers for re-application to the cane fields.

This exciting new development has been in testing at the pilot level for over four years. During this time process feasibility has been firmly established. This year (2002) is the year in which the developers, Tongaat-Hulett Sugar Limited and South African Bioproducts Limited, are planning a large-scale test equivalent to a cane-crushing rate of 1,000 tcpd.

This paper summarizes the process concept, describing the technologies in each of the unit operations, then outlines the expected advantages and finishes by addressing the integration aspects of implementing the WSM process in a cane mill. There are impacts to factory operation in the areas of steam, water and energy demand and usage. It is also necessary to deal with the disposition of the impurities present in the by-product waste streams. This will be treated in more detail at a later date.

Advances in both continuous ion exchange and membrane technologies have been significant in the last decade making economically feasible the present process concept. New WSM installations are not inexpensive but with reasonable sugar price differentials between raw and refined sugar the payback is attractive.

The production of a highly purified juice early in the cane processing scheme will provide sugar millers, in the future, a truly versatile intermediate stream from which to produce product mixes of varying types that fit into the local market needs – crystal sugars, refined products, amorphous powders, fermentation plant feeds etc. A clear high purity juice avoids the special treatments often applied to remelted crude crystals. The WSM technology has the potential to eliminate cane refineries as we know them today.

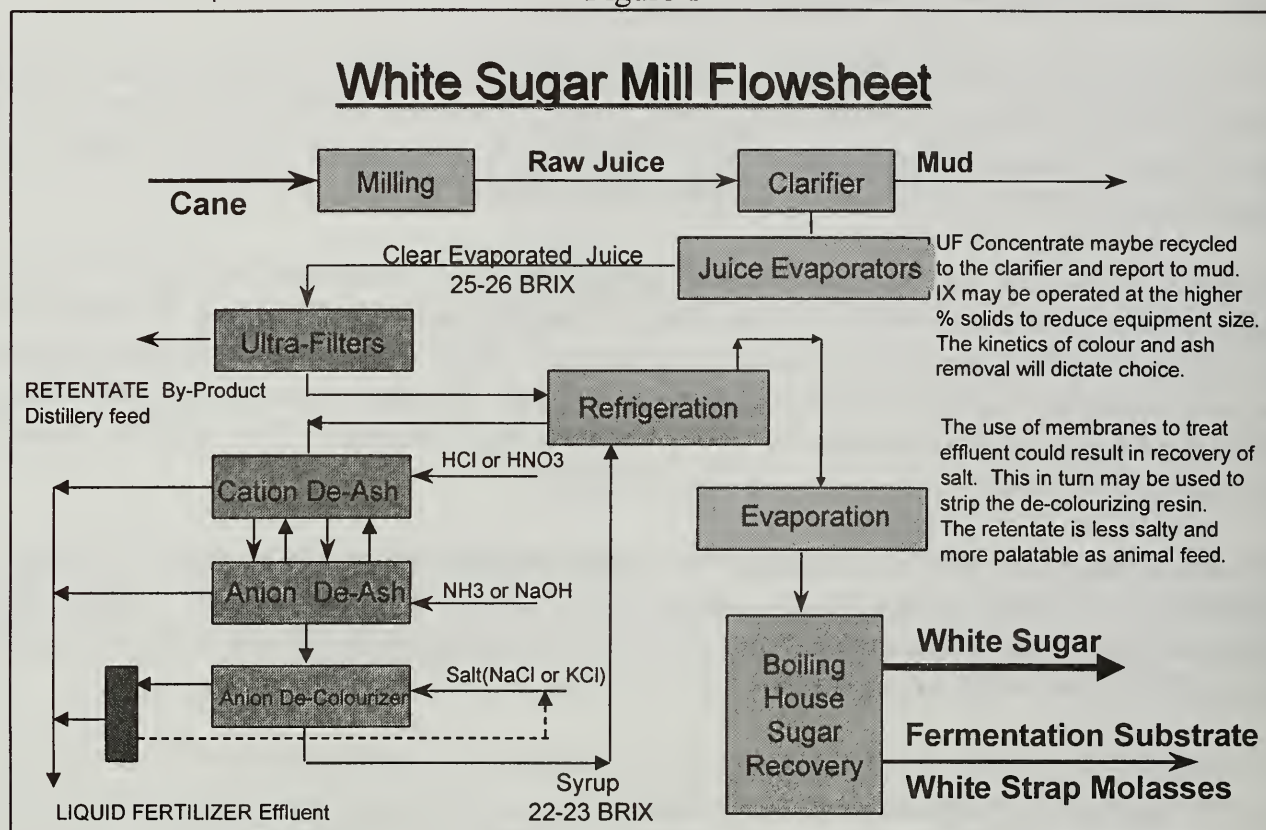
The logic of the process lies in the benefit of no longer requiring a remelting of crystal and the associated energy needed to re-crystallize from affination syrup. Direct juice purification in the mill employs less expensive energy than that utilized in a stand-alone refinery.

WSM PROCESS

Tongaath-Hulett Ltd. and their partner S.A. Bioproducts Ltd. have spent over 5 years developing and piloting this new concept to purify clarified juice in order to make white refined sugar and a high-grade fermentable molasses. The site of the bulk of the work has been the Felixton Mill. Felixton has a nominal crushing capacity of 600 tcph. In a typical year mill production yields 290,000 tonnes of raw sugar. Pilot testing for the last three years has taken place inside the mill facility using live juice during the season and stored juice in the off-season.

The basic process concept is depicted in Figure I. It comprises the operations of Ultra or Micro Filtration followed by Resin De-ashing and De-Colourizing steps. The resin steps utilize a continuous contacting technology called ISEP. ISEP brings with it much efficiency in the use of water and chemicals. ISEP systems are installed operations at the Tongaat-Hulett refinery in Durban and at the Umbogintwini lysine plant of S.A. BioProducts.

Figure 1



The new process can be flexibly applied. The choices of regenerants can be tailored to minimize the impact on the local environment or be chosen to minimize cost. Chemicals for these tasks can be more than 50% of all incremental operating costs. The UF recovery to permeate is chosen to keep investment and power costs to a minimum. The use of de-colourization may be optional in some market environments where high boiling house recovery is not required (Brazil). Fundamentally, the Ultra-filters protect the resins from soluble species that otherwise would foul the adsorbents, seriously reducing their life. The robustness of these membrane units is a critical factor in the economics. The ion exchange de-mineralization step comprises two continuous ISEP units coupled together as an anion-cation pair.

Piloting has demonstrated that the life expectancy of these resins has exceeded that in the original economics. De-colourization follows de-ashing and completes juice purification through adsorptive extraction of organic non-sugar components. The resin steps take place at reduced temperature in order to minimize loss of sucrose to inversion.

To appreciate the juice purity increases, Table I lists the typical profiles that have been consistently demonstrated in the more recent 2001 piloting campaign. Figure 2 gives one a

visual appreciation of the difference between the clear juice feed and the purity of the “White Juice”.

Table 1

	Colour (IC)	Conductivity $\mu\text{S}/\text{cm}$	Turbidity (ICUMSA)	pH	Purity (%)
WSM Feed	25,000	5,000	8,000	6.0	85.0
Permeate	24,000	5,000	500	5.5	85.5
De-ashed Juice	3,500	200	<50	5.0	91.5
WSM Product	<200	<200	<50	7.0	92.0

Figure 2
Samples of the Purified Juice after each stage



The ultra-filtration unit removes turbidity and high molecular weight compounds that will foul ion exchange resins. Colour removal is marginal in ultra-filtration. Calcium and phosphate are partially removed.

De-ashing or demineralization of juice uses cation and anion exchangers operating as a couple. This process takes out over 95% of the mineral content leaving a juice that measures < 200 $\mu\text{S}/\text{cm}$. The de-ashing units also reduce by 80% the incoming colour compounds. A final de-colourization with anion resin completes the colour removal down to levels consistently < 200 ICUMSA units.

The product juice returned to evaporation is essentially a highly pure sucrose solution containing some invert and other higher sugars. It returns at a 10% dilution (clarified juice basis). This exceptional juice purity brings many benefits to the downstream operations.

BENEFITS OF PURIFIED JUICE

The primary economic benefit of WSM comes in the form of product sales:

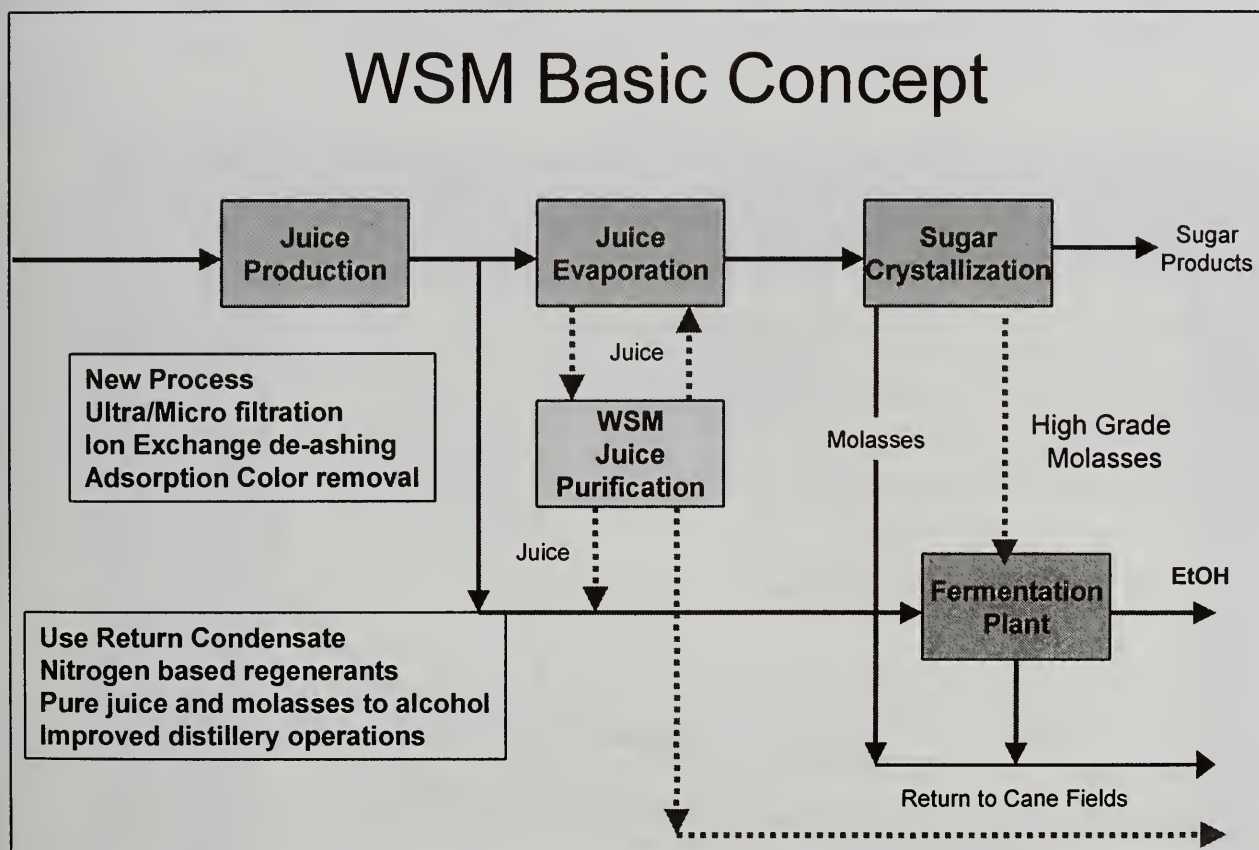
- 1) There is an **increase in sugar yield** that comes from the purity increase in the syrup fed to the pans. This increase is typically 6-8% more sucrose in the bag compared to raw production. To put things in perspective: the A-strike receives a 92 purity syrup and after recovering over 55% crystals the liquor feed to the B-strike is around 85 purity. This liquor purity of the first run-off is similar to that of a conventional mill syrup, however it is less viscous and has a higher invert content than the norm. The pan operations continue from here after already having "bagged" over 50% of the feed sucrose!
- 2) There is a **sugar quality improvement**. Sugar crystallized meets the EEC 2 specification and so commands a premium price that varies from country to country. A typical difference (world market prices) in recent years has been \$30-40 per metric tonne. In some countries the difference can be more than twice these levels.
- 3) As originally conceived for BioProducts, the **production of a high-grade molasses** (coined 'White Strap Molasses') is of immense value as a cheap fermentation substrate. Usually mill or Black Strap sells for a fraction of the value of sucrose contained. This new product has demonstrated very significant yield and productivity improvements in certain fermentation reactions. This arises from the very low ash content, which reduces the osmotic stress on the organism. There is evidence that the remaining impurities can also be beneficial to a fermentation operation.
- 4) It is well known that a purer sucrose feed to the **alcohol fermentation** brings significant increases in yield. For this reason, the alcohol producers have concentrated on improving the molasses quality. **The "white" juice and white strap molasses combined will be a very pure feedstock** and can be the basis for a profitable operation.
- 5) WSM feed is clarified juice pre-evaporated to an optimum brix. This optimum value is that value which maximizes the UF 'brix-flux' and minimizes the ion-exchange plant size (1). In most sugar mills, and it stands to reason, the scaling of heat transfer surfaces occurs once the solubility limits of the various salts and other soluble species are exceeded. This phenomenon normally commences after the second effect and becomes progressively worse all the way to through the crystallization steps, where mineral precipitates contaminate the sugar product. Now, with this pure, ash-free, colour-free, juice we expect **no fouling of these surfaces in the evaporators and vacuum pans**. Heat transfer coefficients (HTCs) will be enhanced and remain close to those one sees immediately after cleaning.

- 6) **Higher HTC's translate into faster heat transfer.** Higher capacity ensues in the later evaporators and also in the vacuum pans. Shorter residence times in any particular stage can result in less thermal degradation and less inversion. The HTC improvement is further enhanced on account of the juice being less viscous. From crystallization experiments with the white syrup, one concludes that it is possible that the A-station cooling crystallizer may no longer be necessary.

WSM IMPACT ON MILL OPERATIONS

Any changes made to an industrial process require some careful thought as to how to integrate them into the existing plant operations. WSM is no exception. Figure 3 shows the place of the WSM operation in the main mill flowsheet. From the process standpoint it is a single black box that receives colored juice and returns a white juice.

Figure 3



Raw clear/clarified juice enters. White juice exits and proceeds through further evaporation and crystallization. In the figure the dotted lines show the main juice and effluent flows from the WSM operation. The impurities leave the process as either liquid salts or as dissolved organics, in one or more effluent streams. In a conventional mill these impurities would report to the molasses by-product stream. The requirements to accomplish this purification are energy in the form of pumping power for membrane filtration and chemicals and water for resin operations. Having purified the juice, improved performance is enjoyed in the downstream evaporation and crystallization steps. The integration questions remain; water management for the unit

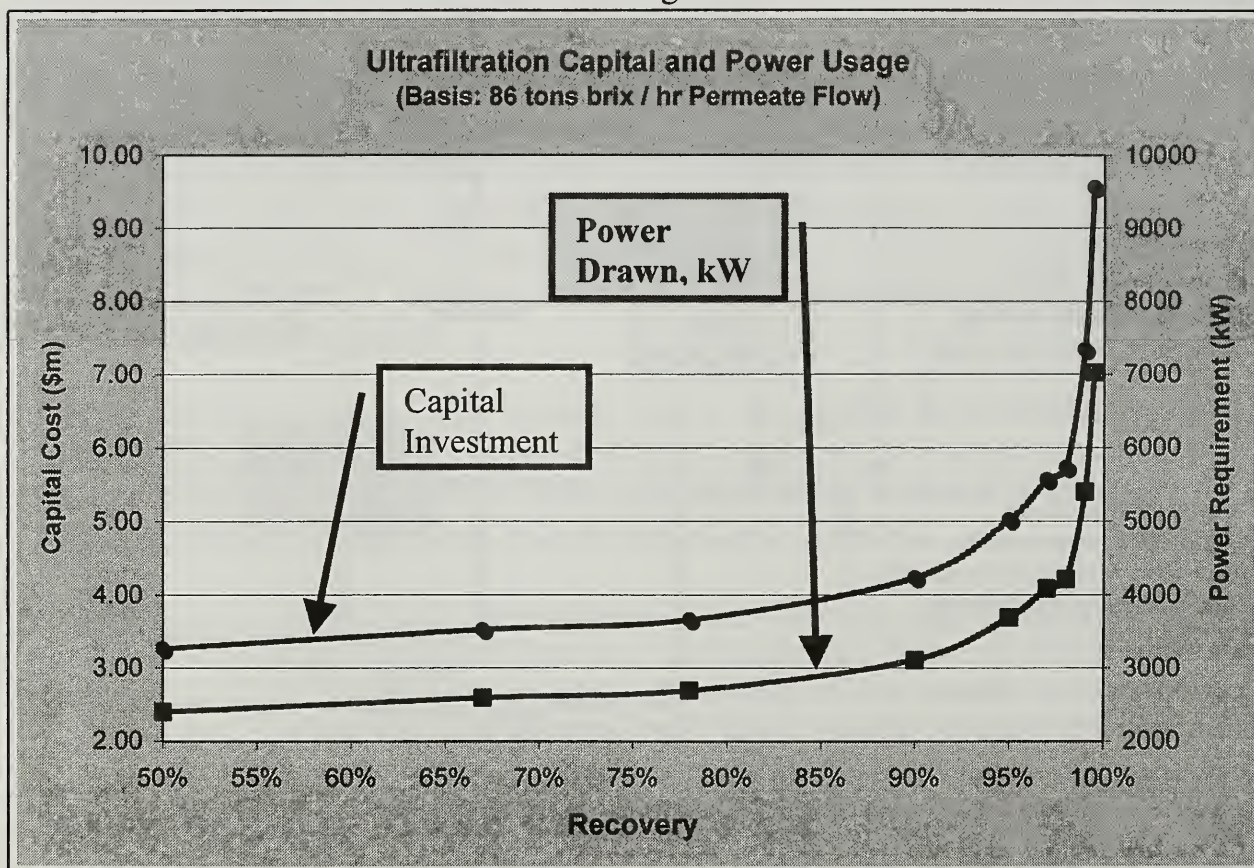
operations of WSM; power requirements for the pumping and cooling systems; effluent stream characterization and treatments.

The process extracts minerals and organic compounds, the vast majority of which originate in the cane plant itself. Having extracted them onto resins they are chemically desorbed as soluble salts. The choice of acid and base to be most compatible with nature means that nitrogen-based chemicals, namely nitric acid and ammonia, are utilized.

UF & IX Pumping Requirements

The ultra-filtration operation is a major new consumer of electrical energy. The UF load will approach 5.4 MW under normal factory operating conditions. The system as designed will recover 99% of the sucrose as permeate and will use some dia-filtration in order to reach this goal. Figure IV estimates the UF capital costs as a function of permeate recovery. It clearly shows that the high permeate sucrose recovery comes at a steeply increasing price (capital and operating) after 90% permeate yield is achieved. Consequently, the choice of operating point may be determined by the current premium price for sugar end products.

Figure 4



The choice of how much UF recovery is optimum, at least when first applying the technology, may well be related to the existing installed turbine capacity. To avoid additional power generating equipment cost a lower permeate recovery is an option. The retentate can be partially or fully recycled. Table II sheds some light on the investment impact of this situation. At the

Felixton mill they are fortunate to already have an extra 10 MW turbine in place. In many places there is an opportunity for power sales and so the added expense of recovering more sucrose (permeate) becomes an economic decision associated not only with sugar premiums but also with the power prices.

In Table 2 the 50% case would apply to plants that divert juice to an on-site distillery. The 90% case would apply where a retentate bleed is of economic value or can be recycled to the extraction section of the mill.

Table 2
UF Costs & Permeate Recovery (mill 600 tcph)

	Capital \$ Millions	Operating MWH/year	POWER MW
UF, 50% recovery	2.21	5156	1.19
UF, 90% recovery	3.70	11748	2.72
UF, 99% recovery	7.19	22831	5.28

It should be pointed out also that cleaning frequency and maintenance when operating at 50% recovery decreases. Lower brix recovery operations also extend the expected life of the membrane units. Beyond 95% permeate recovery water is required for dia-filtration in order to keep power consumption down.

The power-draw of the liquid pumps that service the three ion-exchange units adds up to around 0.7 MW. The ion exchange technology is designed to operate at the kinetic limits of the resin adsorption and de-sorption rates consistent with acceptable bed pressure drop.

As resins become more robust and ISEP equipment handles higher pressures then smaller particle sizes will demand higher flow rates to take advantage of enhanced kinetics. This is a desirable direction to move since it also minimizes residence time in the resin beds, which translates to less inversion in the cation columns. For some incremental increase in pumping power it may be possible to operate without refrigeration and only water cooling while containing inversion to low levels (< 1%).

Juice Cooling Requirements

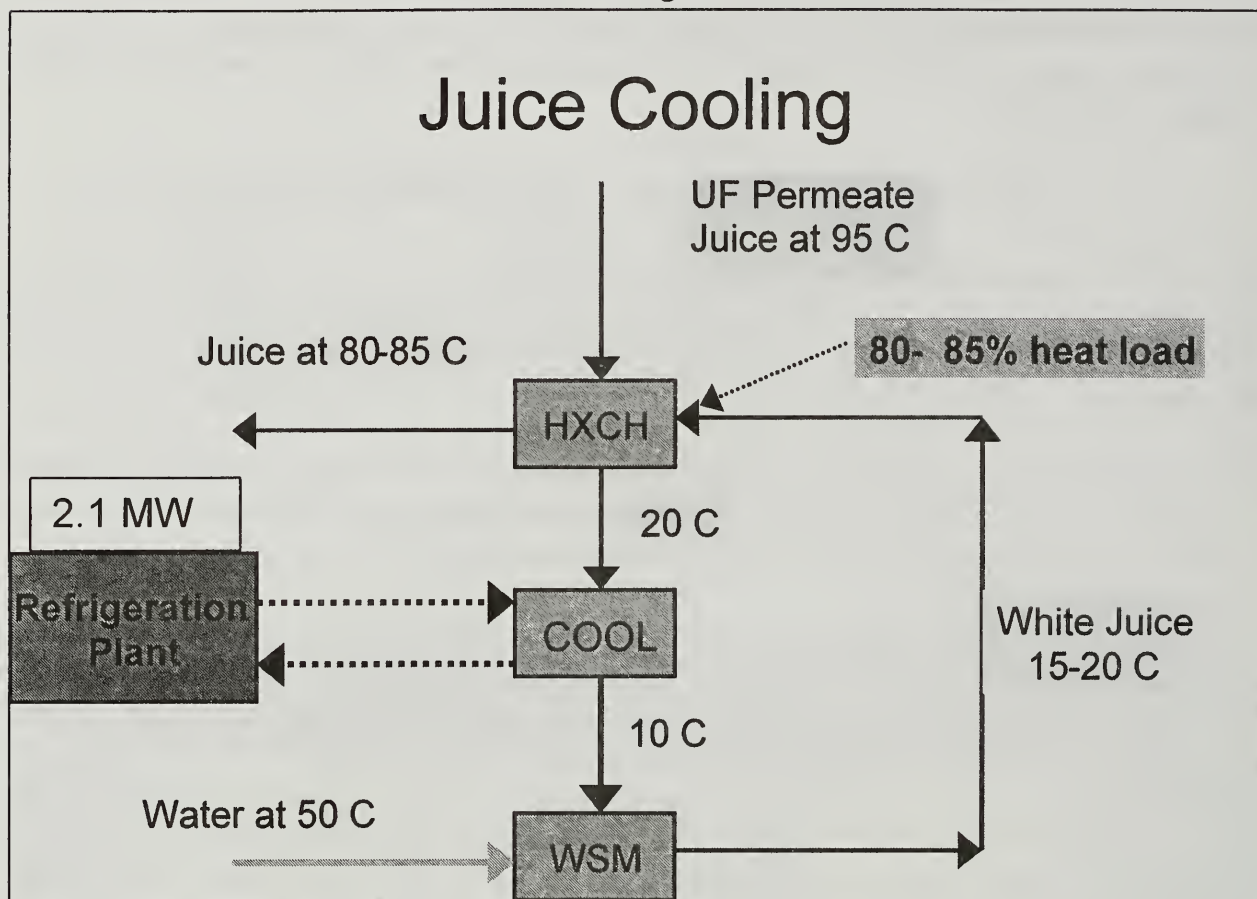
Early in the development programme it became clear that operating the cation exchange process at normal juice temperatures would result in an unacceptable amount of inversion. To keep the sucrose loss to invert to reasonable levels the process needs to operate at temperatures below 35° C. At 15-20° C in pilot operations inversion loss is <0.5%. To guarantee minimum inversion the first plant will be equipped with refrigeration cooling to as low as 10 C.

Figure 5 illustrates the steps of cooling the juice feeding the resin plant and then re-heating the purified juice. To minimize the refrigeration power demand, about 85% of the cooling load is accomplished through heat interchange between feed juice and the product juice. This approach also minimizes the juice re-heating requirement.

The decision on whether to include the refrigeration investment and its operating overhead will again be a local decision. For instance, where some loss to inversion is of no great concern, such

as in Brazilian mills that feed the molasses to alcohol plants, then this part of the new plant can become a fortunate irrelevance.

Figure 5



The question has arisen concerning the microbiological implications of operating at lower temperatures. No obvious degradation has taken place in all the pilot runs so far. The juice is well pasteurized upon arrival at the WSM battery limits. The juice storage buffer is for 60 minutes after having been cooled prior to ion exchange. From juice storage it has no more than a 15-minute residence time before it is reheated to 90+ C.

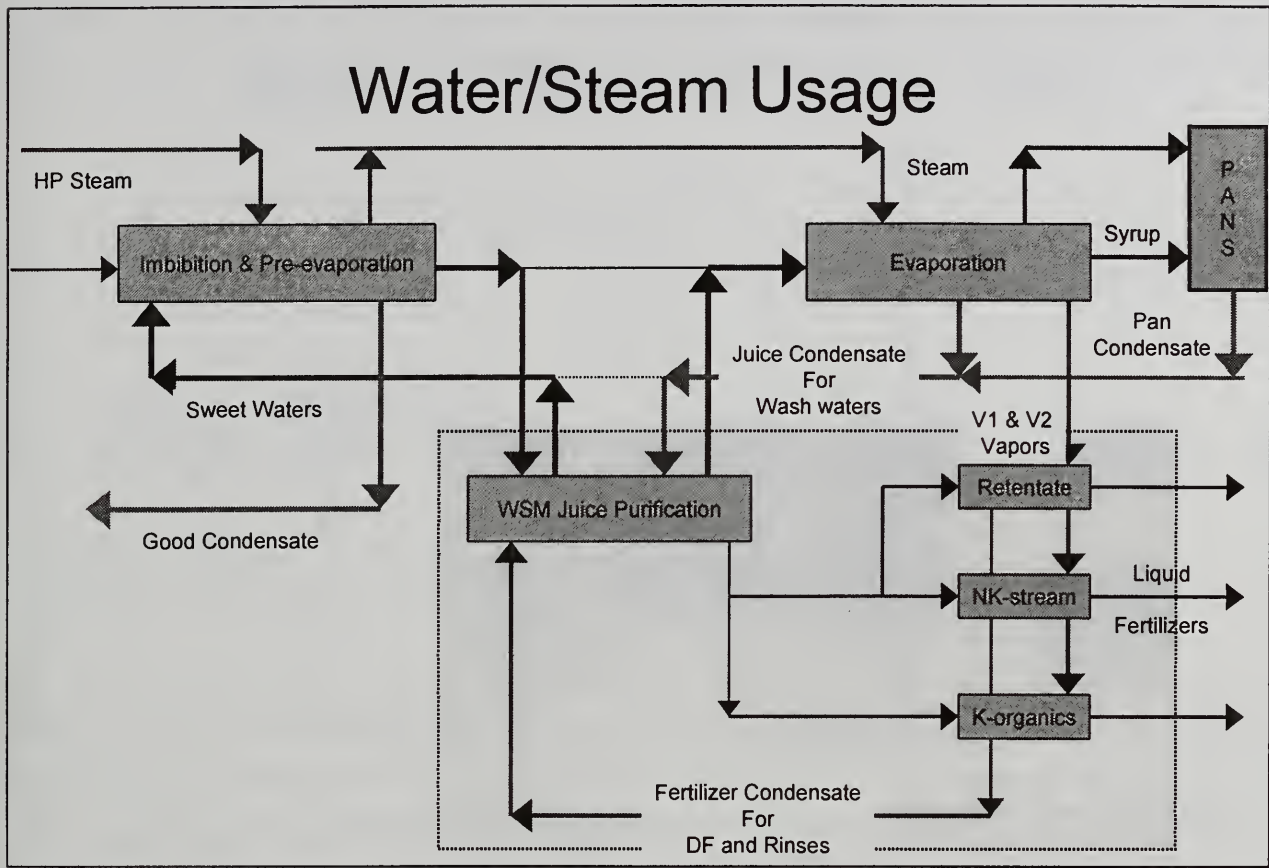
Water Usage Considerations

The WSM operations employ water for dia-filtration (when necessary) and resin washing and rinsing steps. The ISEPs use significant quantities of water although the technology is so flexible that rather elaborate water economy schemes are easily implemented in a steady state manner.

Figure 6 illustrates the major flows of water and steam moving into and out of the WSM process. Return condensate from juice evaporators and the vacuum pans normally returns to imbibition. Now some of that water is first used in the ISEP equipment resin washing steps and is then returned as sweet water to imbibition. The dilution of the juice is approximately 10% based on water in the un-evaporated clear juice flow. For a 600 tph mill the water loss to the combined

concentrated effluents amounts to around 18 tph. Table III, below shows typical water flows for a 600-tcph mill.

Figure 6



From the data in Table 3 it is also clear that there is no shortage of juice condensate water to satisfy ion exchange and dia-filtration needs. The pressure operated 1st effect's condensate will naturally be returned to the boiler feed system.

Table 3
Typical Water flows – 600 tcph

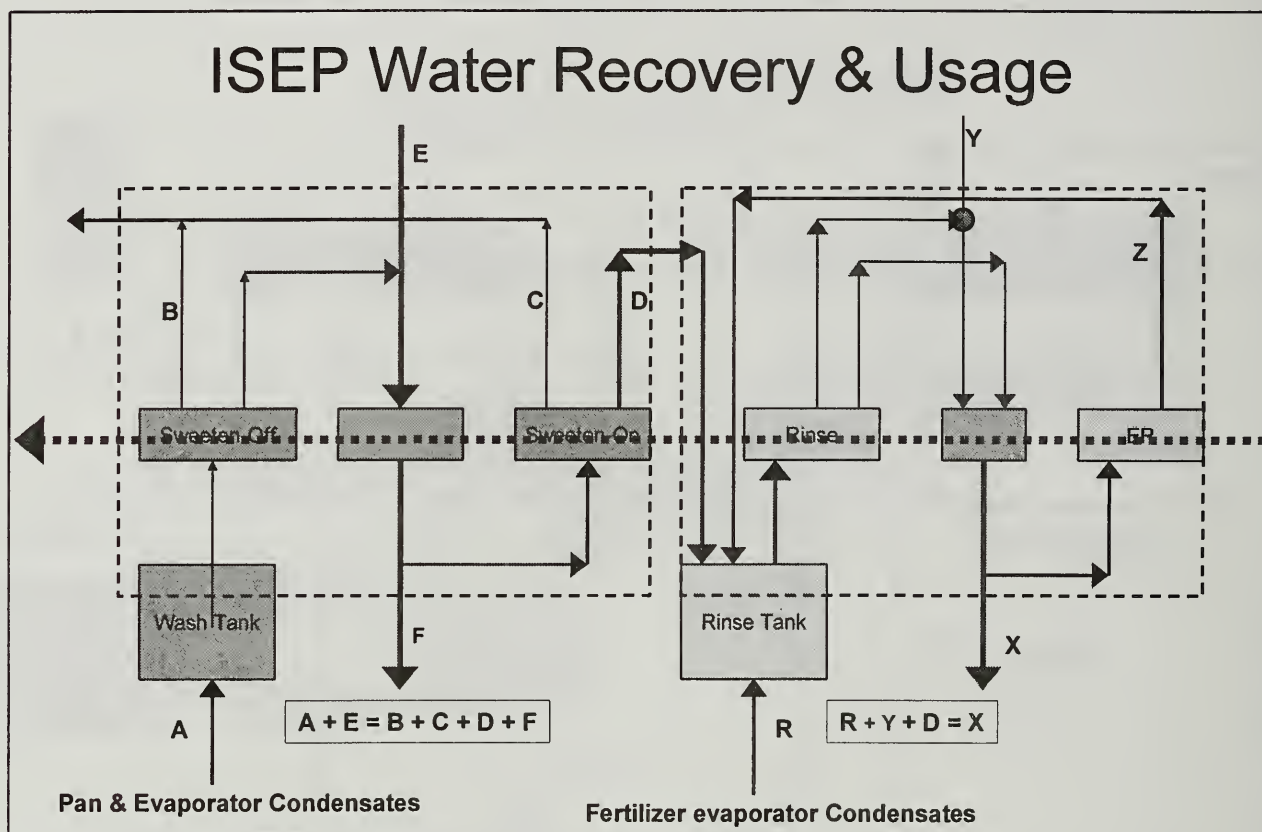
Stream	tph	
Juice Condensate available	330	Juice condensate from effects 2-5
Dia-Filtration demand	30	Use Juice condensate or salt condensate
IX wash demand	84	Use juice condensate
IX rinse demand	166	Use salt condensate and supplement with juice
Effluent Condensate available	154	Condensate from V1 or V2 vapours

The basic water management philosophy follows the following rules,

- 1) Maximize resin sweet water recycle to imbibition and so minimize juice dilution
- 2) Use **juice condensate for resin washes** – in this manner any leakage of wash liquor into the juice will not cause any contamination. Such contamination could occur when using condensate return from salt concentration operations.
- 3) Use **salt/effluent evaporator condensates for resin rinses**. This rinse water is also used to dilute the concentrated eluents (chemicals) used for regeneration. Consequently we do

not wish to use juice condensate since we risk losing sugar. There is a slight shortfall (see Table 2), which can be made up from juice condensate.

Figure 7



A detailed diagram in Figure 7 shows the various flows for handling the water balances in any one of the three ISEP units in WSM. The two mass transfer zones are flanked by an elaborate configuration of water washes, rinses and water recovery steps. These zones in an ISEP are operated as counter-current plug-flow displacements. End points are detected and controlled by conductivity measurements.

So for instance, in the “Sweeten-Off” zone, a resin bed enters full of clarified juice. The juice is displaced by the counter-current wash flow and is returned into the juice interstage tank. Displacement flow continues until the column effluent conductivity indicates that most of the juice has been pushed out. Juice conductivity is considerably higher than that of condensate wash water. At the point when conductivity reaches a designated value, a valve is activated to switch the column effluent to a sweet water destination (imbibition). A similar state of affairs takes place in the rinse and sweeten-on zones. In the rinse after eluent is pushed out and returned to the elution zone, then dilution water is directed to a system where concentrated eluent requires dilution. Implementing these complex water management schemes on a system with the flexibility of the ISEP valve is quite simple.

Steam Consumption Considerations

WSM causes as is expected a net increase in energy over a conventional mill. We assume that a conventional mill has a steam generation capacity in its bagasse in excess of that required by the WSM process. The question is whether the existing plant has the required electrical capability and/or the necessary installed boiler capacity.

Table 4
WSM Energy & Steam (600 tonnes cane per hour)

	Power, MW	Steam, tph
Turbine power, UF	5.3	70
Turbine power, IX	0.7	9
Steam Turbine, Refrigeration	2.1	22
Vacuum Pans		<50>
Juice Dilution, dilution water		13
Effluents, minimum steam demand		36
Effluents, maximum steam demand		56

One MW generated by a back-pressure turbine translates into about 13-14 tph steam load. Whereas a MW for direct or prime movers can be 9-11 tph HP steam. Looking at Table 4 we see that the pan house savings offset the effluent concentrators. It remains to be seen what quality of steam the effluent units require. The electrical and refrigeration loads will demand some 100-120 tph HP steam. This then means less exhaust steam resulting from less water to the de-superheater now that we are routing more steam through the turbines.

The small amount of steam economy required to handle the dilution ($66/4 = 16$ tph, or $66/5 = 13$ tph) can probably be engineered into the operation by re-configuration of the evaporator trains. Often plants have spare evaporators due to frequent heat transfer surface cleaning. Now these evaporator bodies are freed up for continuous use. So we can turn a quad-train into a 5-effect and a 5-effect into a 6-effect.

Some brief calculations employing a simple model gave the results tabulated below in Table 5. The results take into account the reduced demand from pans and the extra steam needed for effluent concentration.

Table 5
Steam Rates (tph) (plant 600 tcph)

No. Effects (stages)	4	5	6
White Sugar Mill	307	288	273
Raw Sugar Mill	284	271	

Based on these rough calculations one can see the imbalance caused by the demands of WSM if we keep an existing configuration. On the other hand, upon re-arranging the available evaporators, the added stage (effect) to the WSM evaporator train brings us back to the original

consumption. This includes bleed adjustments for feeding V1 vapour to the effluent evaporators and less V2 vapour to the pans.

One of the benefits from a lower syrup viscosity will be that now a higher brix syrup can be delivered to the pan house operations. Pan operations become more efficient on account of the higher purity, lower ash and lower colour of the feed syrup. Expectations are that the first strike, A-station will not only have high yield but may also not need to utilize the cooling crystallizer. Crystal growth rates increase, movement water consumptions go down and there is less re-melting (crystal recycle) required. Table 6 provides us an indication of the old and new pan-house performance parameters.

Table 6
Typical PanHouse Comparison

	Raw Sugar Boiling House	White Mill Boiling House
Feed Brix	68	70
Feed Purity	85	92
Feed Colour	25000	360
Crystal colour	1037	28
% Yield sucrose	89.3	96.0
Molasses purity	38.1	31.7
Steam consumption, tph	91	43
No. Product boilings	1	2.5
No. Re-melted boilings	2	1.5

These energy savings are to be expected and they translate directly into a credit of vapour consumption. The savings here can be used to feed the effluent stream evaporation trains. Each mill will have its own set of circumstances and will require a detailed study.

UF Retentate and Resin Plant Effluents

This is an area where considerable effort has been made to move away from the original eluents and also minimize the volumes of effluents. A future publication will go into more detail on the options available to implement the process in different economic and environmental circumstances.

The first contemplated plant at Felixton, KwaZulu-Natal province in South Africa has the luxury of being able to safely dispose of salt waste. Consequently, the first chemical regenerants tested were HCl and NaOH. All early data showed them to be perfectly compatible with a good de-ashing and de-colourization performance.

Once it became clearer that value could be added to these effluents if they had soil nutrient qualities then other candidate chemicals were tested. The Ion exchange effluents contain the ash, mineral and organic compounds that dissolve into the juice. They are from the cane field and so in principle should be compatible if returned. In addition the effluents contain the regenerant chemicals added to the elution zones. Now if these are nitrogen and potassium based, then they supplement the K and N already in the ion exchange eluates.

For the last season and during the 2002 pilot campaign the resin operations will use HNO_3 and NH_3 , producing an effluent mix of ammonium and nitrate salts. Switching from NaCl in decolourization to KCl will convert that effluent to a predominantly potassium containing solution.

Table 7
Effluent Nutrients

	Composition Kilograms/Tonne cane	
	No salt Recovery	Salt Recovery
Potassium, K_2O	2.86	1.41
Nitrogen, N	1.83	1.83
Organics	7.29	7.29

Table 7 summarizes the main nutrient values in the effluent streams, which will be concentrated up to 50% solids in evaporators. The solids load for the Felixton case is around 13 tonnes/hour without salt recovery. Trials are in progress to look at the compatibility of these effluents used as fertilizers in cane agriculture. Initial results are positive.

Flowsheet Variations

This aspect of the technology is a subject for more depth at a later date. As the application of the process is studied for various countries and milling environments the flow sheet integration into the factory will have to deal with situations such as:

- a) In Brazil many plants in the Sao Paolo state region split the juice flow after clarification and often 50% of the sucrose goes directly to the production of alcohol. Studies need to be made to see what the benefits are to fully or partially purifying the juice stream. So, the cost-benefit of designing for either 50% or 90% permeate and splitting much of the juice after purification need to be assessed.
- b) Many plants still operate with sulphitation in order to produce a low colour crystal. Sulphitation puts calcium and sulphur ash into the juice increasing the ash load. WSM will run well with or without sulphitation.
- c) Mills that feed other fermentation operations do not need to be concerned about some loss of sucrose to invert. In such cases the requirement for complete de-ashing disappears as does the need to operate at very low temperatures. These constraints once removed reduce investment costs for ion exchange (faster kinetics – smaller units) and eliminate the need for refrigeration. These operations may wish to permit potassium to remain in the juice as a nutrient for the fermentation operation.
- d) Plants with back-end refinery plants can now simplify or eliminate the re-melting and refining operations producing savings in steam and plant. A partial application of the process could be interesting in mills where the steam balance is tight. A pure syrup from the mill can be sent directly to affination in a BE refinery situation.

- e) An advantage to this type of plant is the fact that in countries where the season is short, then during the off-season the plant can run as a conventional refinery, processing re-melted raw sugar.
- f) Operating the process without the de-colourizer for those plants that do not require production of EEC 2 crystal specification.

SUMMARY & CONCLUSIONS

The applicability of the WSM process continues to show real promise as a future mill process. The technology to purify clarified juice has been proven during a 4-year pilot campaign that still continues. A larger trial is planned for this year at a scale of 1,000 tcpd. The fundamental basis for the process lies in the logic that purifying the sugar syrup prior to crystallization eliminates the energy and equipment used in multiple re-melting and re-crystallizations necessary today to arrive at white sugar quality crystals. Such a processing option is now available following advances achieved in both membrane and resin contacting technologies during the last decade.

Integration of WSM into an existing mill brings its own set of challenges and they can be classified into three areas. First the impact on the factory operation through water, steam and energy balance changes. Second, the disposition of the effluent streams containing the extracted impurities that previously reported to the molasses. Third, in cases where raw sugar is not the only mill product, adapting the juice operations to meet the requirements of the final products.

In regard to energy, calculations indicate that the power drawn by the new process requires that more live steam be directed to drive turbines. The UF and IX operations are a significant new electrical load amounting to 6 MW in the case of the 600-tcph mill. While a 2MW direct drive compressor will supply power for refrigeration. The need for cooling may be waived in some production situations where loss of sucrose to invert is not a serious economic hardship. The consequence of more turbine let-down is a decrease in steam flow to the by-pass valve and therefore less water addition to remove the superheat. This change reduces exhaust steam to process by a small amount.

The steam usage needed to re-concentrate resin plant effluents is a new energy draw but is largely offset by economies in the pan-house where significant reductions occur. In a situation where back-end refining operations exist the steam used in these operations can be reallocated to juice and effluent water removal. Elimination of scaling in the post-WSM evaporators allows us to re-configure the available bodies to create an additional effect, which further economizes on steam duty.

Effluents are becoming less a waste problem and more an economic by-product that returns nutrients and minerals to the cane fields they came from. The process development has shown that nitrogen based chemicals are not only feasible but desirable for the ion-exchange operations.

Finally, adapting the process to other sugar production environments is a challenge being looked into now. The Brazilian alcohol situation runs parallel with S.A. BioProducts original desire for a high quality molasses. The benefits of purer juice and molasses are being assessed for alcohol fermentation.

One last note to mention that based on early analysis, the process economics are extremely attractive where seasons are long > 160 days and/or where refined sugar premiums over raw sugar are greater than US\$50/tonne.

ACKNOWLEDGEMENTS

I do not claim to be an engineer of any experience at all with regard to sugar mill operations and so I owe so much to fellow authors Craig Jensen & Wolfgang Fechter who both have been a true mentors in this area of technology application. Special thanks to David Love who spent considerable time and energy teaching me enough about evaporation and sugar boiling to be dangerous.

It is also fitting to remember Peter Brewer, the young engineer, who in 1997 or even earlier had the foresight to realize that this process was a worthy objective for his company in its search for a cost competitive raw material to feed fermentation based production plants.

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UPDATE ON THE USE OF HYDROGEN PEROXIDE AT CENTRAL EL PALMAR REFINERY

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ABSTRACT

According to Paper # 618 presented at the 1991 SIT congress in New York by Fernando Cordovez, the use of ion exchange resin columns is the obvious complement to the application of Hydrogen Peroxide in the removal of color from sugar liquors. Nevertheless, this suggestion was not immediately put into action at Central El Palmar (Venezuela) due to both the elevated capital cost and the fact that, at the time, the sugar produced was within the quality standards of the Venezuelan market.

Up to 1993, sugar marketing and distribution in Venezuela was controlled by a monopoly. With the dissolution of the monopoly and the subsequent opening of the domestic sugar industry, quality requirements were heavily increased. Particularly high standards were set by the soft drinks industry. There was stronger emphasis on color reduction, and ash and sediment content. Market requirements motivated the installation of an ion exchange resin station at Central El Palmar, Venezuela.

This paper gives an account of the decolorization station; describing procedures used and results obtained. A station of ion exchange acrylic resin columns replaced the initial decolorization process by H_2O_2 . This station was further complemented by the installation of cation exchange columns, used for softening. Nonetheless, The need to eliminate poorly or non-ionised colorants suggested the use of H_2O_2 as a complement to the resin station, but in a smaller dosage than previously applied. Proper functioning of this station requires the elimination of hydrogen peroxide residuals to prevent resin damage. The decolorization station used today is described, presenting the color transfer factor(CTF) reduction achieved.

Among the conclusions, it was found that the combined use of H_2O_2 and ion exchange resins in the decolorization station allows the refining of sucrose that achieves the soft drinks industry standards on a regular basis. The reduction in CTF gives proof of the effectiveness of the combined decolorization method. Lastly, the replacement of acrylic resins by styrenic resins is suggested due to the low color obtained after the process of clarification by phosphatation-peroxide.

ABOUT CENTRAL EL PALMAR

Central El Palmar S.A. (CEPSA) is a cane sugar mill with an attached refinery located near the city of San Mateo, Aragua State, in Venezuela. It is located in the valleys of La Cordillera de la Costa, a mountain range that spans the north of Venezuela. Its first crop season took place in 1956 with a milling capacity of 3600 metric ton/day.

Nowadays, CEPSA has a milling capacity of 9000 metric ton/day during the crop period, lasting from late October to early May. The average production during this period is of 110,000 metric tons of refined sugar from 1,200,000 metric tons of sugar cane. There is also a second production period, from June to late October, called the off-crop period. During these months approximately 80,000 metric tons of raw sugar, imported mainly from Colombia and Central America, are refined, with average pol ranging from 97.5 to 98.5.

CEPSA processes close to 700 metric tons/day of raw sugar, through a process of affination and dissolution to a Brix of 65. Hydrogen peroxide is simultaneously added to the product, with a pH controlled between 7.0 – 7.2. The sugar then undergoes a process of clarification by phosflotation. The chief filtration is through Tate & Lyle deep bed filters. The final decolorization of the filtered liquor is completed at an ion exchange resin station, composed of cation resin (used for softening) and acrylic resin (used for decolorizing).

DECOLORIZATION STATION BACKGROUND

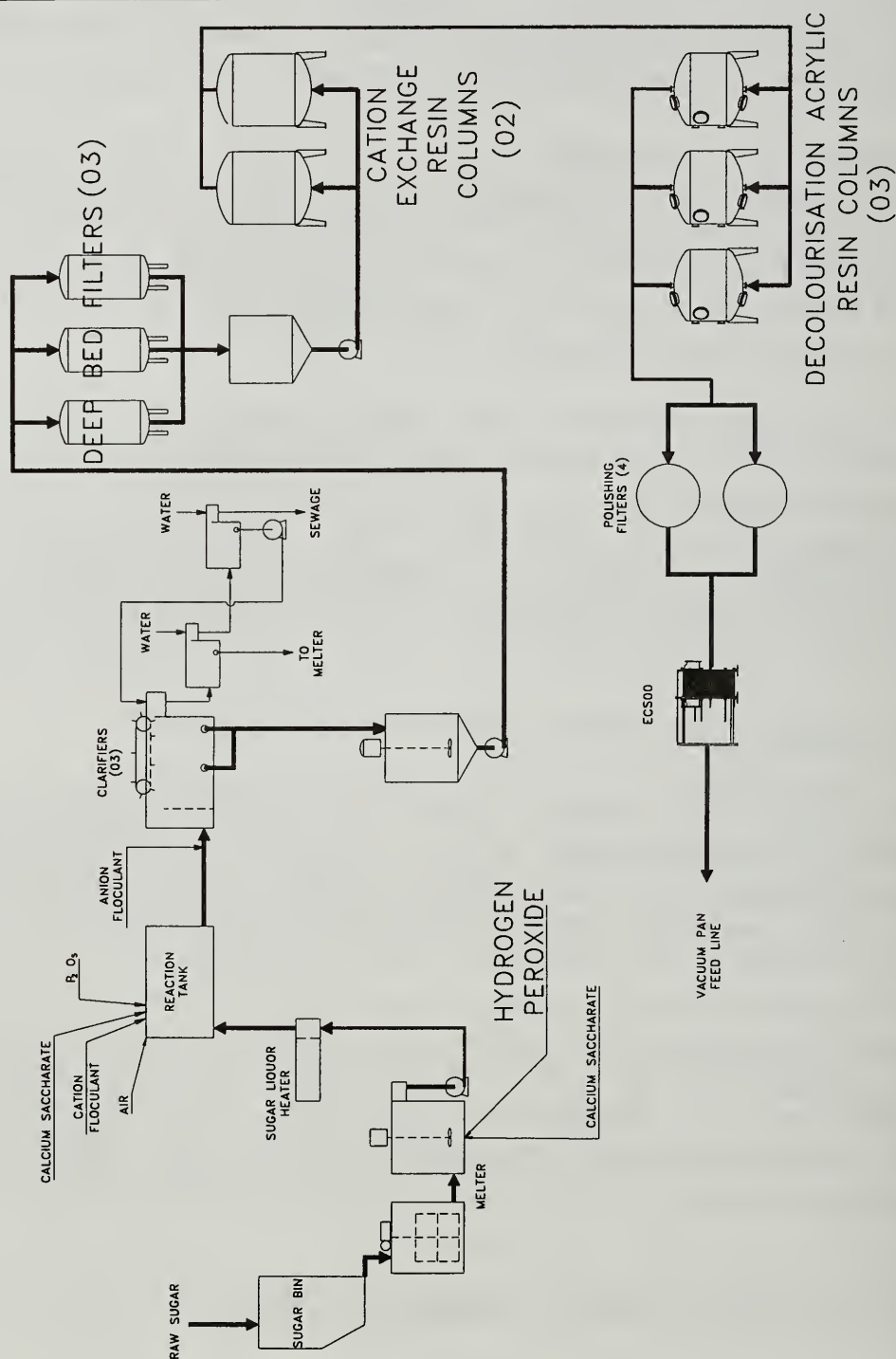
During the 1991 SIT congress in New York, Fernando Cordovez et al. (1) presented a paper about the refining process used at the time in CEPSA. Back then, the chief decolorizing agent was hydrogen peroxide in a 50% solution. Cordovez et al. (1) suggested at the end of that paper the use of ion exchange resins as a complement to H_2O_2 . This suggestion, however, required too great an investment at the time. Nevertheless, in 1993 the DVA (Venezuelan Sugar Distributor) was dissolved, opening the domestic sugar market (as reported by F. Vollmer at the 1996 SIT Packing Symposium). This enabled each refinery to commercialize its sugar production independently and without the intervention of the Venezuelan government. In 1996 CEPSA decided to invest in a decolorization station, using acrylic ion exchange resins. The motivation for this investment was the potential ability to supply refined sugar that could meet the quality requirements needed by certain clients, which were above the quality standards in Venezuela.

INITIAL DECOLORIZATION STATION

The initial ion exchange resin column station became operational in June 1997. It consists of three units, one always set to stand-by or undergoing preventive maintenance. Each unit contains 10.5 m^3 of resin.

The liquor, filtered to a Brix of 65 and at 70°C , flows through the columns of I.E. in up-flow. The resin used is a strongly basic anion resin. The resin is made of a polyacrylic base with a macroporous structure, in the chloride form.

REFINERY FLOW CHART



The system utilized in CEPESA is semi-automatic. Each column, upon completing one cycle, enters a regeneration phase, according to the following procedure:

1. De-Sweetening: The liquor is displaced with hot water in downflow, for approximately one hour.
2. Regeneration: Accomplished with a solution of 8-10% sodium chloride (10 °Be), and 1.5% sodium hydroxide. The amounts of each compound are 200g and 15g, respectively, per litre of resin. This translates to 23,000 litres of 10 °Be flowing downward in a period of 1-hour (6.4 l.p.s.).
3. Slow Rinsing: Performed immediately upon conclusion of the regeneration stage. Its purpose is to eliminate any remnants of brine inside the unit. This stage has duration of one hour.
4. Rapid Rinsing: With a duration of 20 minutes and a volumetric flow rate of water of 15 l.p.s., also in downflow.
5. Sweetening: Water is displaced in upflow with liquor at 1.5 bvh, headed to the reservoir vessel for the vacuum pans.

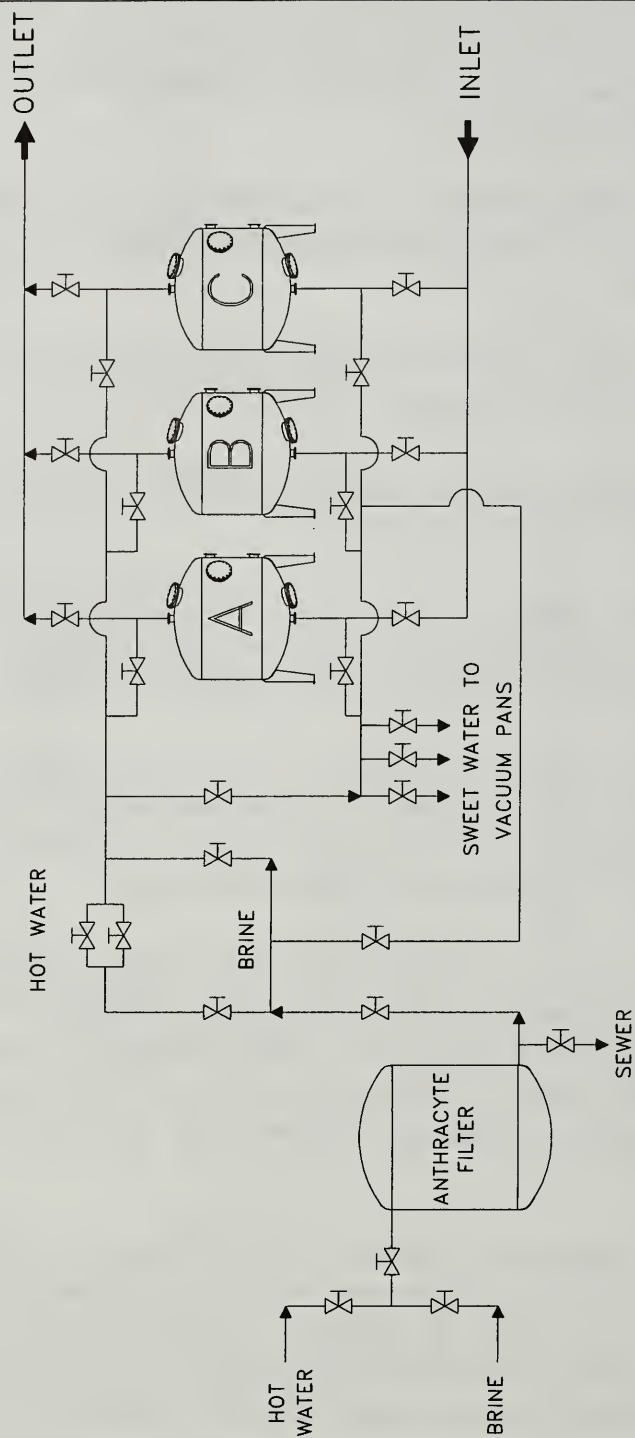
During the first 100 cycles of operation (24-36 hours per cycle), the ion exchange resin columns yielded an average rate of decolorization of 65% with inlet liquors ranging between 400-600 IU. The decolorizing effect subsequently dropped to an average of 50%.

By the time the resin had reached 250 cycles of operation, the % decolorization had dropped below normal levels. Together with a purchase order for a new batch of resin, it was decided to add hydrogen peroxide to the phosflotation treatment. This was done with the intention of augmenting the % decolorization of the final liquor.

The addition of H_2O_2 presented CEPESA with a new challenge: to prevent the filtered liquor that feeds the ion exchange resin columns from having any residual H_2O_2 . The presence of highly oxidant compounds, such as H_2O_2 , would damage the structure of the resin. Thus, the quantities of H_2O_2 added were far smaller than those of previous procedures, as described earlier, and always carefully eliminating any residual hydrogen peroxide with the use of sodium hydrosulphite.

The process began with addition of 150-200 ppm in a 50% solution, reaching up to 250-350 ppm. The first batch of resin operated for approximately 435 cycles per column, when it was replaced by a new batch. In addition, CEPESA began the installation of two columns of 8 m³ of volumetric capacity, filled with cation resin. One of the columns would operate in series with the existent units and the other one would be either set to stand-by or undergoing preventive maintenance.

INITIAL ION EXCHANGE DECOLOURISATION RESIN STATION



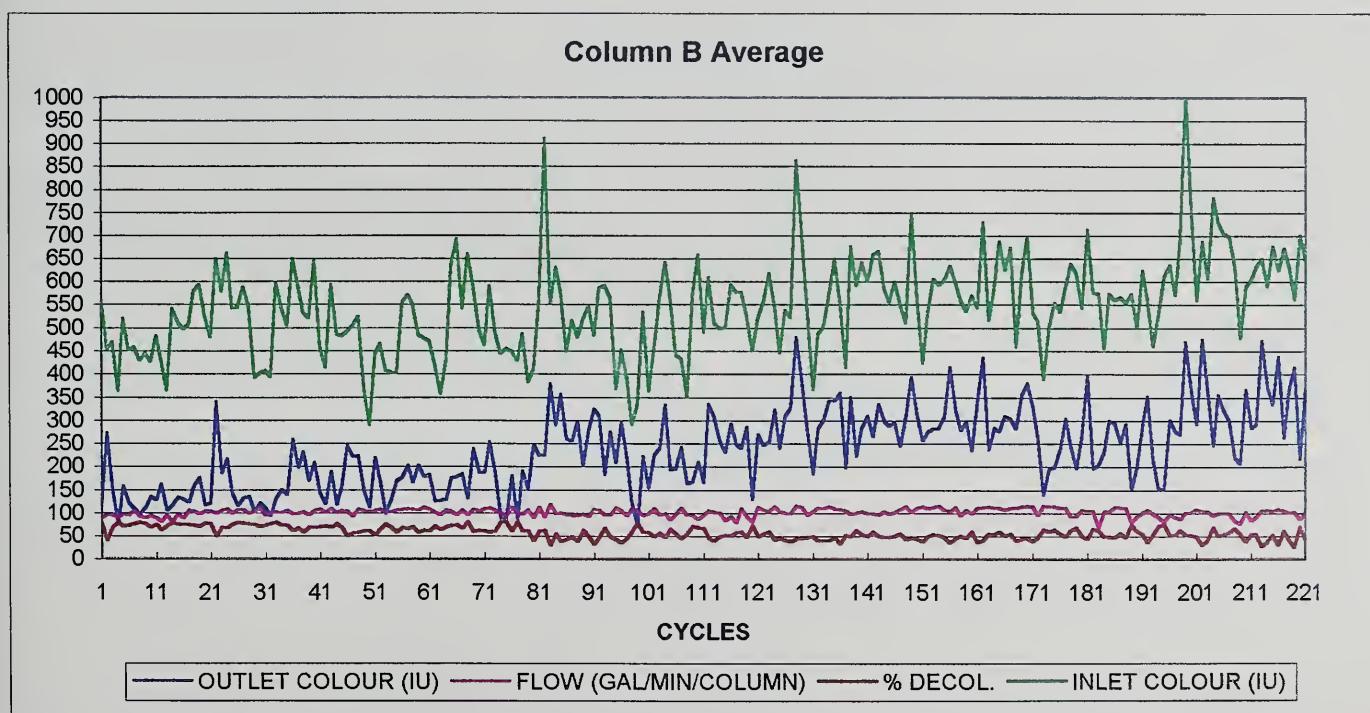
The cation exchange resin was incorporated into the process with the sole purpose of removing any hardness in the liquor (softening). This would avoid having to rinse the vacuum pans due to solid incrustations and thus, ultimately increasing the efficiency of the decolorization columns.

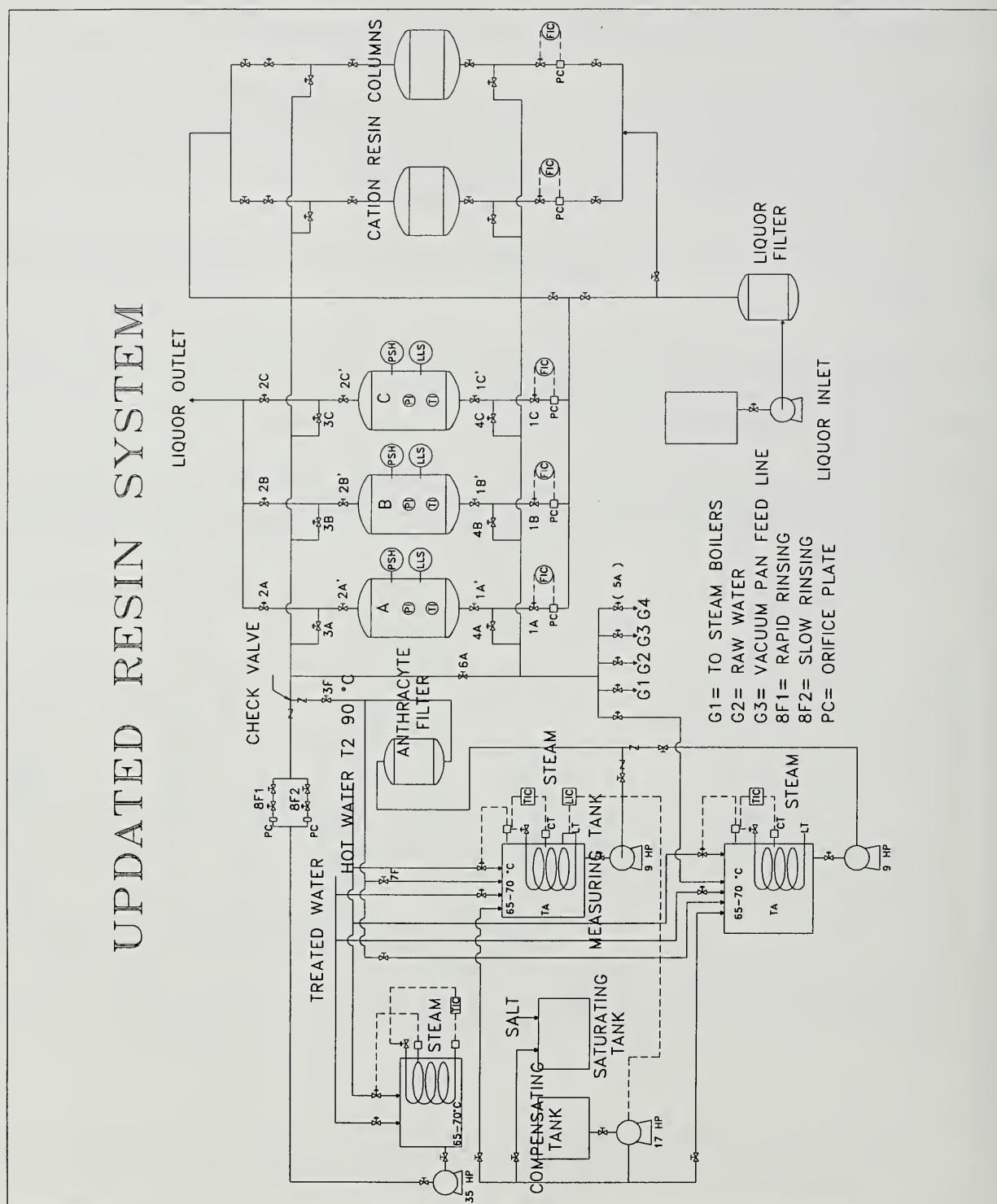
COLORANTS IN THE PRODUCTION OF SUGAR FROM SUGAR CANE

The diversity and heterogeneity of the colorants involved in the production of sugar make this aspect of the process rather complex. Nevertheless, it is possible to divide the colorants into two groups: those coming naturally from the raw material (sugar cane), and those formed during the process, by chemical and thermal destruction of sugars.

According to some authors quoted by Cordovez et al. (1), five general groups of colorants have been identified:

1. Melanines, products of oxidation and condensation of 3,4-dihydroxyphenylalanine (dopa).
2. Colored products from the alkaline degradation of sucrose.
3. Caramel-type colorants, produced by excessive and prolonged heating of glucose, sucrose and fructose.
4. Melanoidins, produced by the Maillard reaction between amino-acids and reducing sugars.
5. Iron-polyphenol complexes of high color, formed by combinations of iron and polyphenols, such as dopa.





These five groups were arranged by Riffer (3) in an ascendant manner by molecular weight and in a descendant manner by IV. The behaviour of each colorant group with respect to the sugar crystal in the production process is as follows:

During the refining process of raw sugar, certain reactions take place that alter the nature of the coloring agents inherently present within the sugar. According to the author (3), during the elaboration of raw sugar, there is predominance in the occlusion of large molecular weight - pH insensitive coloring agents (low IV) to the surface of the sucrose crystal, when compared to the small molecular weight – pH sensitive coloring agents (high IV). However, the opposite effect takes place during the refining process. This is explained by the large concentration of coloring agents with low IV and large molecular weight in the components of raw sugar.

Different stages during the refining process, chiefly affination, induce the changes mentioned at the beginning of the last paragraph. These stages enable the predominance in the concentration of colorant agents having low molecular weight and high IV, which tend to be occluded in the sugar crystal. Phenol colorants, inherently present in sugar cane, represent the vast majority of the colorant agents in question. They are most effectively removed by conventional decolorization methods, mainly bone char and other absorbents.

The same author (4) states that hydrogen peroxide oxidizes the color forming compounds into intermediate compounds, which are non-reactive and unable to form color. H_2O_2 can also break unsaturated bonds and diketones present in the colorants, forming carboxylic acids. Lastly, the author states that hydrogen peroxide oxidizes phenols to produce quinones and non-cyclic compounds. Kofold-Nielsen et al (5) suggest hydrogen peroxide oxidizes colorants to produce melanins, which can be removed during the process of clarification of dissolute particles.

Hydrogen peroxide has little reactivity to color at a pH <7.0. At a pH <6.5, H_2O_2 tends to increase the level of complexes formed with metals such as iron, neutralizing its decolorizing action. Thus, H_2O_2 is applied at a pH level controlled between 7.0 and 7.2.

APPLICATION AND RESULTS

When the original batch of resin was nearing the end of its useful life, a few important clients began to demand an improvement in the color of the final sugar. This motivated the conduction of the first applications, as previously described in this paper. Much attention was paid to ensure that no residual H_2O_2 was present at the inlet of the resin columns. The positive results attained transformed this procedure into a routine check.

The dosage is applied at the main melter, together with cation color precipitants and sufficient calcium saccharate to maintain a pH level of 7.1. Then follows the conventional treatment of phosphatation and clarification of the liquor by flotation. In this treatment, the reaction pH level is controlled in such a way as to be able to attain clarified liquor with a pH of 7.2.

Comparative results about the application of H₂O₂, together with the decolorization due to ion exchange using acrylic resin, can be seen on the following table:

Year	1996	1997	1998	2000	2001
Treatment	H ₂ O ₂ Only	Acrylic Resin Only	Acrylic Resin Cation Resin (Softening)	Acrylic Resin + Cation Resin And Addition of H ₂ O ₂	
Sugar from Affination	1014.00	962.00	1055.00	1061.00	944.00
Liquor to Vacuum Pans	448	370	371	401	310
% Dec	55.8	61.5	64.8	62.2	67.2
Color in Refined Sugar	75	54	57	52	35
Color in A Refined Sugar	49	38	37	35	22
Global CTF %	16.74	14.59	15.36	12.97	11.29
A Sugar CTF %	10.8427	9.4525	9.9730	8.7282	7.0968

**Color Transfer Factor (CTF)
Reduction**

Average

% Red. GCTF vs. H ₂ O ₂ only	0	-12.82	-8.22		-27.55
% Red. GCTF vs. Acrylic Resin only		0	5.30		-16.87
% Red. ACTF vs. H ₂ O ₂ only	0	-12.80	-8.00		-27.01
% Red. ACTF vs. Acrylic Resin only		0	5.53		-16.27

Other Quality Parameters

YEAR	1996	1997	1998	2000	2001
	H ₂ O ₂ only	Acrylic Resin Only	Acrylic Resin Cation Resin (Softening)	Acrylic Resin + Cation Resin And Addition of H ₂ O ₂	
SUGAR					
REDUCING SUGARS	0.017	0.014	0.012	0.011	0.011
ASH	0.025	0.020	0.015	0.012	0.011

DISCUSSION

The apparently modest decolorization % is due to the inability of the deep bed filters to guarantee proper filtration during periods of poor clarification. This allows undesirable solids to leak into the columns, frequently restricting the flow of liquor through them. In order to maintain the pressure drop at an acceptable value in the resin station, the fraction of the liquor flow that cannot go through the columns must altogether by-pass the decolorization station. As the two liquor flows reunite downstream of the resin station, the colorants present in the by-pass flow show in the color of the final liquor. Hence the seemingly modest values.

Nonetheless, the CTF for A Sugar was reduced by 27% when compared to the treatment using H₂O₂ only and by 16% when compared to the treatment using Acrylic Resin only. A similar

trend is logically followed by the Global Color Transfer Factor (GCTF), but with slightly larger percentages. The results suggest that the hydrogen peroxide, in the indicated dosage, is eliminating phenol-type colorantss. Acrylic resin is incapable of eliminating these phenolics, due to the inherently low ionising % of these colorants and the lower absorption capacity of the resin, at the pH the resin is exposed to in the refinery.

Mane et al. (6) quotes that H_2O_2 is selective when it comes to the elimination of color. It attacks melanoidins and caramel-type colorants chiefly. Nevertheless, the results from our controlled pH low dosage applications (<300-ppm) demonstrate that H_2O_2 also reacts notably against phenol-type colorants, which are more likely to be occluded in the refined sugar crystal. Thus, the color transfer factor drops notably.

Other parameters such as ash % and reducing sugar % in refined sugar were drastically improved. This is owed chiefly to the use of the softening (cation) resin and the careful pH control at the location of H_2O_2 addition. It would be far too daring to give the peroxide alone the credit for this improvement.

A conventional filtering station would represent the obvious solution to the filtration problem, but a rather costly solution. Besides, a filtering station also keeps latent the danger of filter aid leakage to the columns, and the recurring cost this generates. Other potential solutions include the use of softening cation resin in downflow, together with clarification improvements. This would trap the undesirable solid leakage from the deep bed filters in the downflow columns, to be subsequently eliminated with backwash. Experiences quoted by Alder et al. (7) back up this solution, which is less costly than a filtering station and also serves the dual function of softening and filtering.

The quality of the results obtained in decolorization % during the clarification by H_2O_2 suggests the use of styrenic resin in lieu of acrylic resin. This proposal would significantly improve the decolorization effect, given the aforementioned filtration problem is resolved beforehand.

CONCLUSIONS

- The described refining scheme enabled CEPASA to meet the standards required to supply an important part of the market for bottling of soft drinks in Venezuela.
- The effectiveness in the use of hydrogen peroxide as a complement to the acrylic resin station is demonstrated with the 16% reduction in the CTF to the sugar crystal, with respect to the use of acrylic resin only.
- Attention must be paid to the meticulous elimination of residual hydrogen peroxide in the liquor that feeds the resin columns, in order to guarantee the structure of the resin.

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HYDROGEN PEROXIDE AS A PROCESSING AID IN THE CANE FACTORY

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INTRODUCTION

SPRI has ongoing research to look at ways to improve the quality of sugar during processing. In this program, we have been particularly interested in juice clarification. Clarification is a crucial stage in raw sugar production, and its efficiency has a dramatic impact on the rest of the process, including factory efficiency and final raw sugar quality. For this reason, mixed juice clarification should be as effective as possible. Additionally, with the dual trends toward producing edible sugar at the factory and the desire to cut down on usage of chemicals, such as SO₂ for clarification, the need to find alternative methods is critical.

Membrane Filtration

SPRI has been studying membrane filtration and ultra filtration for the last few years. For many years ultra filtration has been examined as a clarification process in the cane sugar industry, but has not yet been applied commercially. However, ultra filtration appears to be a quite good clarification process for raw juice, especially in terms of removal of turbidity and macromolecules. However, color removal by membranes, an important factor in the quality of clarified juice, is low. An alternative means of reducing color in conjunction with membranes is needed.

Hydrogen Peroxide

A review of the literature has shown that hydrogen peroxide has been used with success for some years in several countries as a decolorizing agent in the sugar industry, in refineries.

Hydrogen peroxide is a strong oxidizer (corrosive at high concentration), with the aspect of water and a slight acidic odor. It is a non-polluting chemical with a short period of life, decomposing into water and oxygen. FDA has classified it as Generally Recognize As Safe (GRAS). Hydrogen

peroxide has a long history of use in industries such as paper and water treatment, cosmetics manufacture, where it is mostly used as a bleaching, disinfecting and deodorizing agent.

It was decided to combine the ultrafiltration qualities of mineral membranes, which are resistant to hydrogen peroxide and considered a safe food processing aid, with the decolorizing potential of hydrogen peroxide for cane juice processing.

EFFECT OF ULTRA FILTRATION AND HYDROGEN PEROXIDE TREATMENT ON CANE CLARIFIED JUICE

The first phase of the study was to check on a laboratory scale if a high quality juice could be obtained in terms of turbidity, macromolecule and color content, the critical parameters to produce a good quality sugar. Three membrane cut-off levels were tested (15 kDa, 300 kDa and 0.1 μm) in combination with two different hydrogen peroxide concentrations (350 and 500 ppm, w/v) on clarified cane juice. The juice was adjusted to pH 8 with sodium hydroxide before hydrogen peroxide addition, in order to maximize its action.

The second part of the study consisted in verifying that hydrogen peroxide did not lower the sucrose content.

Part One: UF/H₂O₂ experiments

Materials

Filtration was performed with a Kerasept UF lab scale pilot. This pilot contains one membrane only and is equipped with a manual valve on the retentate outlet which allows increasing the system pressure to compensate for gradual membrane fouling. Mineral membranes were used. The clarified juice used for the experiment was provided by a cane mill in Louisiana. Thirty percent chemical grade hydrogen peroxide was used.

Procedure

The juice was adjusted to pH 8 at room temperature (22° C), the hydrogen peroxide added, and the juice heated to 85-90°C, then ultrafiltered. During ultrafiltration, the retentate was recirculated to the feed while the permeate was collected. The experiment was terminated either when the membrane was fouled or when all the feed was treated.

To determine repeatability, each membrane cut-off/H₂O₂ combination was repeated three times. We also ran three tests for each membrane cut-off level with untreated juice (no H₂O₂), to determine the membrane effects alone.

Results and Discussion

The values presented below are the average of three tests. Color, turbidity, dextran and polysaccharide were analyzed in the feed, retentate and permeate. Table 1 presents color and turbidity removal by membrane filtration alone and Table 2 shows the color removal when H₂O₂ is added as a membrane pre-treatment.

Table 1. Color and turbidity removal from juice by membrane alone.

Membrane cut-off	Turbidity Removal (%)		Color Removal (%)	
	Avg	Coef Var (%)	Avg	Coef Var (%)
15 kDa	90.7	9.0	13.9	9.0
300 kDa	93.1	4.0	18.2	31
0.1 µm	96.5	3.0	12.5	55

As expected, the ultrafiltration treatment was highly efficient for turbidity removal (>90%) but it showed a low capacity for color removal (~15%). There was also a high variability in color removal, especially with the higher cut-offs.

This variation in color removal is also noted in Table 2, which shows the color removal by ultra filtration combined with hydrogen peroxide. Hydrogen peroxide had a dramatic and constant effect on color removal. The removal in color was 24-25 % at 350 ppm and 31-32 % at 500 ppm at each membrane cut-off, showing that the hydrogen peroxide was responsible for the major color reduction, and not the membrane. This experiment demonstrated the potential of hydrogen peroxide for removing a significant amount of color from the clarified juice by oxidizing the color compounds (see Figures 1 and 2).

Table 2. Effect of hydrogen peroxide on color removal (%) in the ultra filtered juice.

Sample	Membrane	Membrane + H ₂ O ₂		% Increase
		Avg	Coef Var (%)	
15 kDa / 350 ppm	13.9	37.9	16	24.0
15 kDa / 500 ppm		46.3	13	32.4
300 kDa / 350 ppm	18.2	42.0	34	23.8
300 kDa / 500 ppm		49.7	23	31.5
0.1µm / 350 ppm	12.5	37.5	24	25.0
0.1µm / 500 ppm		43.7	10	31.2

Figure 1.

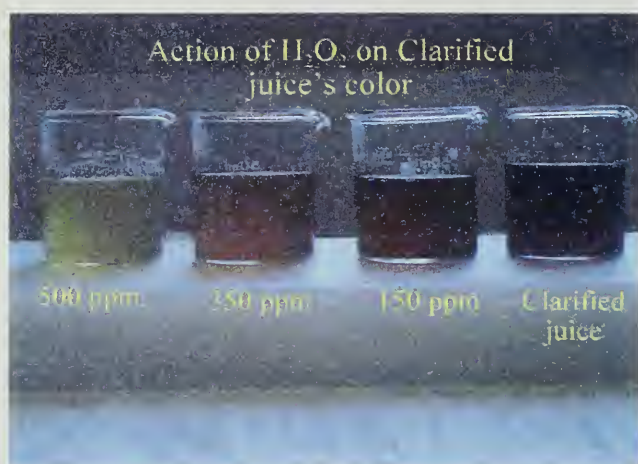
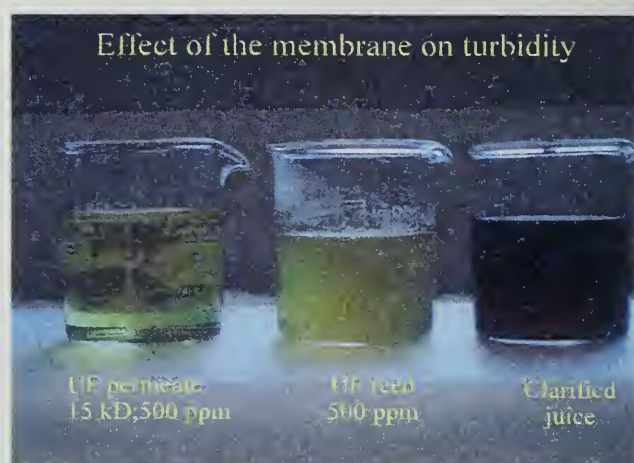


Figure 2.



Macromolecules. The clarified juice used in these experiments had a very low starch content, so only total polysaccharide and dextran were analyzed. As shown in Table 3, ultrafiltration removed significant amounts of polysaccharide and dextran (~35-60%), with the larger amounts removed by the lower cut-off.

Table 3. Removal of macromolecules by ultra filtration

Membrane cut-off	Polysaccharide		Dextran	
	Avg (%)	Coef Var (%)	Avg (%)	Coef Var (%)
15 kDa	59.2	3	57.1	14
300 kDa	48.1	4	43.9	10
0.1 μ m	35.7	11	39.8	5.2

Hydrogen peroxide had no effect on polysaccharide removal (Table 4) or dextran (Table 5). The results were within $\pm 5\%$ of the results for membranes only (Table 3).

Table 4. Polysaccharide removal (%) by H_2O_2 + ultrafiltration.

Sample	Membrane alone	Membrane + H_2O_2		% Change
		Avg (%)	Coef Var (%)	
15 kDa/350 ppm	59.2	57.9	7	- 1.3
15 kDa/500 ppm		56.8	14	- 2.4
300 kDa/350 ppm	48.1	53.0	0.07	4.9
300 kDa/500 ppm		52.5	0.16	4.4
0.1 μ m/350 ppm	35.7	-	-	-
0.1 μ m/500 ppm		35	0.41	- 0.5

Table 5. Dextran removal (%) by H_2O_2 + Ultra filtration.

Sample	Membrane alone	Membrane + H_2O_2		% Change
		Avg (%)	Coef Var (%)	
15 kDa/350 ppm	57.1	52.5	0.23	- 4.6
15 kDa/500 ppm		51.4	0.11	- 5.7
300 kDa/350 ppm	43.9	40.8	0.16	- 3.1
300 kDa/500 ppm		45.6	0.07	1.7
0.1 μm /350 ppm	39.8	-	-	-
0.1 μm /500 ppm		40.9	0.6	1.1

Part Two: Effect of H_2O_2 on Sugar Content / Optimization of H_2O_2 Action

Besides process efficiency, the other major objective was to determine that hydrogen peroxide had no negative effects on sucrose content. For that purpose, tests were conducted on clarified juice to check the effect of heat, pH adjustment and H_2O_2 , alone and combined, on the purity. We also studied the effect on color of H_2O_2 alone on clarified juice as well as the influence of $\text{Ca}(\text{OH})$ for pH adjustment to more closely duplicate factory practice.

Effect of H_2O_2 on sugar content

Materials and Procedure

The same procedure as described above were used: pH adjustment to 8 with $\text{Na}(\text{OH})$, H_2O_2 injection followed by heating. The treated samples were analyzed on HPLC for sucrose and/or invert content.

Analytical Results and Discussion

The effect of pH adjustment, heat and hydrogen peroxide on sugar content in clarified juice was tested separately and combined. As shown in Table 6, neither heat nor pH adjustment or both together caused significant variations in either glucose or fructose content (standard deviation < 2%).

Although a purity drop is observed in Table 7, hydrogen peroxide treatment of clarified juice doesn't significantly affect the sucrose and invert content. This statement is confirmed by the consistent results in Table 8 and by the fact that the purity drop in Table 7 is only 2%, which is low and may be linked to analytical error or to slight variations in experimental procedures.

Table 6. Effect of heat and pH adjustment on sugar content in clarified juice.

Samples	Brix	Glucose (% solids)		Fructose (% solids)		Invert (% solids)	
Feed	13.8	1.44		1.67		3.11	
heat #1	14.8	1.37		1.65		3.02	
heat #2	14.5	1.35		1.57		2.92	
heat #3	15.2	1.35		1.61		2.96	
		M: 1.36	Sd: 0.01	M: 1.61	Sd: 0.02	M: 2.97	Sd: 0.02
Feed	13.7	1.45		1.53		2.98	
pH #1	12.6	1.45		1.57		3.02	
pH #2	13.5	1.47		1.58		3.05	
pH #3	13.5	1.42		1.53		2.95	
		M: 1.45	Sd: 0.02	M: 1.56	Sd: 0.02	M: 3.01	Sd: 0.02
Feed	13.7	1.45		1.53		2.98	
heat-pH #1	13.4	1.46		1.53		2.99	
heat-pH #2	14.6	1.44		1.54		2.98	
heat-pH #3	14.5	1.4		1.54		2.94	
		M: 1.43	Sd: 0.02	M: 1.54	Sd: 0	M: 2.97	Sd: 0.01

M = average Sd = coefficient of variation

Table 7. Effect of H₂O₂, heat and pH adjustment on clarified juice.

Sample	Brix	Sucrose (% solids)	Glucose (% solids)	Fructose (% solids)
Feed	12.94	87.55	2.03	1.97
350 ppm #1	12.34	88.73	2.16	2.13
350 ppm #2	11.12	85.95	2.16	2.18
500 ppm #1	12.26	85.18	2.18	2.15
500 ppm #2	11.54	85.87	2.18	2.14

Table 8. Effect of 500 ppm H₂O₂, pH adjustment and heat treatment on sucrose and invert content in clarified juice.

Samples	Brix	Sucrose (%solids)		Glucose (% solids)		Fructose (% solids)		Invert (%solids)	
Feed	13.7	86.45		0.79		1.34		2.13	
S #1	14.8	86.25		0.79		1.38		2.17	
S #2	15.2	86.87		0.82		1.21		2.03	
S #3	14.9	86.03		0.82		1.15		1.97	
S #4	14.2	87.67		0.80		1.28		2.08	
		M:	Sd:	M:	Sd:	M:	Sd:	M:	Sd:
		86.7	0.01	0.81	0.02	1.26	0.08	2.06	0.04

Optimizing the action of H₂O₂

Materials and Procedure

The same general procedure as for H₂O₂ + ultrafiltration was used: Clarified juice was adjusted to pH 8 with either NaOH or Ca(OH)₂, heated to 85-95° C and hydrogen peroxide added at 500 and 1000 ppm (w/v) concentration. Each experiment was repeated three times with the same clarified juice.

Analytical Results

The results are presented in Tables 9 and 10, showing the average values of three experiments.

Table 9. pH adjusted with Na(OH)

Samples	Color	
Feed 500 ppm	11,861	
Product 500 ppm	6656	Coef Var 3.3%
Removal	43.9 %	Coef Var 4.2%
Feed 1000 ppm	10,392	
Product 1000 ppm	5438	Coef Var 5.6%
Removal	47.6 %	Coef Var 6.1%

Table 10. pH adjusted with Ca(OH)_2

Samples	Color	
Feed 500 ppm	10,392	
Product 500 ppm	6236	Coef Var 0.1%
Removal	40 %	Coef Var 0.2%
Feed 1000 ppm	11,861	
Product 1000 ppm	5430	Coef Var 2.4%
Removal	54.2 %	Coef Var 2.0%

The results of color removal obtained with Ca(OH)_2 are in the same range as those obtained with Na(OH) , with less than 10% difference between them.

Conclusion

To summarize, the use of hydrogen peroxide as a processing aid combined with ultra filtration for color removal in clarified juice is quite successful with, on average, 32% color decrease at 500 ppm (w/v) concentration and very little or no sucrose degradation. The combination of membrane filtration with hydrogen peroxide on a lab scale produced a very high quality clarified juice, with low color, very low macromolecule content and nearly no turbidity. Such a high-grade juice should facilitate the rest of the process and produce a better quality raw sugar as well as a potential edible product.

IMPROVEMENT OF SUGAR CRYSTALLIZATION BY ULTRA-FILTRATION AND HYDROGEN PEROXIDE TREATMENT

As shown in the results above, the overall quality of clarified juice was largely improved by hydrogen peroxide addition (30 to 50% color removal) associated with ultrafiltration treatment (90 to 100% turbidity removal and 50% polysaccharide and dextran removal).

The next phase was to use the high quality treated clarified juice to produce, on a lab scale, sugar to determine the quality improvement in the crystalline sugar. Another objective was to observe the storage stability of the produced sugar.

Experiment #1: Materials and Procedure

The same raw juice described earlier was treated with the 300 kDa ultrafiltration and 500 ppm H_2O_2 combination. Six batches of ultrafiltration permeates were combined and evaporated under vacuum to a 60-65 brix syrup which was boiled and crystallized in a lab scale vacuum boiling pan. The

crystals were separated from the massecuite in a batch lab-scale centrifugal. The sugar was stored for 75 days.

Results and discussion

Color

Table 11 presents the color analysis of the various materials. The hydrogen peroxide and ultrafiltration combination removed 35% color and 90-100% turbidity (turbidity data are not shown). The produced raw sugar color of 626 ICU represents 95% removal of the initial color. The crystal size was small due to difficulty controlling the small pan temperature and the stirring speed, and optimized pan conditions may well have led to a better result. The color transfer observed from syrup to sugar was about 10%, about the same as seen in industrial situations, which could probably be improved by optimizing the boiling conditions.

There was a slight but significant color reduction (6%) after juice concentration to syrup. In mills, there is typically an increase in color (Iqbal et al, 2000). The color decrease on evaporation was attributed to the all-glass system and ideal evaporation conditions (constant vacuum and low temperature); there may also have been some residual hydrogen peroxide, but this is unlikely, given the long evaporation time and the fact that hydrogen peroxide is unstable at higher pHs and to agitation. In a factory situation, an excess of hydrogen peroxide would not be desirable because it is corrosive to metal, and iron salts will cause a great deal of color formation in the presence of hydrogen peroxide (Riffer, 1988). If hydrogen peroxide corrosive action is inevitable, it is possible to remove the colored iron complex by adding sodium hydrosulphite (Cordovez et al, 1991).

Table 11. Color analysis.

Sample treated with 300 Kd membrane and 500 ppm H₂O₂

Sample	Color (Icu)		Removal (%)
	Average	Coef Var	
Feed *	11,325	3.6%	-
Permeate *	7378	3.1%	34.5
Syrup	6691	-	40.9
Sugar	626	-	94.5

* average values of the six batches.

Color on Storage

However well the sugar produced has been washed while in the centrifugal, a small layer of molasses remains surrounding the crystals. Our system is not yet optimized for centrifugal washing. After centrifugation, the sugar was dried and stored in glass containers, in a controlled temperature room. After 75 days the color of the sugar had increased from 626 Icu to 702 Icu, a 17% increase. We are

continuing to study the effect of storage on sugars produced in this manner.

Macromolecules

Table 12 shows the effect on total polysaccharides and dextran after the combined hydrogen peroxide/ultrafiltration treatment.

Table 12. Removal of macromolecules (Values are the average of 3 tests)

Sample	Polys, ppm	% Removed	Dextran, ppm	% Removed
Uf Feed	6118	---	3081	---
Uf Permeate/syrup	2162	64.7	1101	64.3
Sugar	303	86.0	136	87.6

Transfer of polysaccharide into the crystal from the syrup was 14% and dextran was about 12%. This represents an improvement over the 30-50% of dextran and polysaccharide that has been observed to transfer into raw sugar under normal factory operations (Godshall and Baunsgaard, 2000). The same authors had observed a 33% transfer of the polysaccharide in raw sugar into the refined crystal. Another study had shown approximately 25% transfer of polysaccharide from raw juice into raw sugar across the season in Louisiana (Iqbal et al, 2000).

Purity

Table 13 presents the sucrose and invert results for the feed, permeate and sugar. Hydrogen peroxide did not promote degradation of sucrose, as evidenced by the unchanged glucose and fructose contents.

Table 13. Sucrose and invert content.

Sample	Sucrose (% solids)	Glucose (% solids)	Fructose (% solids)
Feed	75.38	1.30	2.14
Permeate	84.68	1.35	2.02
Sugar	99.9	0.08	0.07

Experiment #2: Materials and Procedure

In this experiment 500 ppm (w/v) of hydrogen peroxide was added to the clarified juice and it was ultra filtered through a 300 kDa membrane. Three batches of treated clarified juice were combined and evaporated following the same technique as in experiment #1, to obtain a 60-65 brix syrup.

This set of experiments was conducted on a new boiling pan with a larger capacity (3 liters) which allowed for better control of both temperature and vacuum. The heating was performed by a double jacketed system fed by a water bath.

For each experiment, the pan was loaded with 2 kg of syrup. The syrup was first heated to 80° C for 30 minutes in order to melt all nuclei that may be present in the solution. It was then evaporated until the saturation brix was reached (70° C; 24-25 in). Once the saturation brix was reached, the vacuum was cut and the seeding performed. A seed slurry from a Louisiana factory was used. The seed was mixed with the syrup at atmospheric pressure at a temperature no higher than 70° C. The seeded syrup was then boiled at 23-25 in and 70° C (+/- 5° C) until the crystals reached the desired size or until the massecuite became too tight to be stirred. The vacuum was adjusted during the experiment in order to maintain a good boiling which enhances the stirring and hence the rate of crystal growth. The massecuite is then discharged. Before the centrifugal stage, we keep stirring the massecuite for a moment, while heating, to allow the crystals to grow larger. The centrifugal stage is performed with the same material and in the same way as in experiment #1.

Results and discussion

The results of the UF+H₂O₂ treatment on the clarified juice were in accordance with those obtained in prior experiments as shown in Table 14, with average removal of 41.5% color and of 34.8% polysaccharides.

Table 14. Effect of UF and H₂O₂ on color and macromolecules in clarified juice.

Samples	Color (Icu)	Polysaccharide (ppm)
Feed/batch 1	12,075	5042
Permeate/batch 1	6223	2448
Removal (%)	48.5	51.4
Feed/batch 2	11,441	3226
Permeate/batch 2	7219	2643
Removal (%)	36.9	18.1
Feed/batch 3	10,579	4121
Permeate/batch 3	6446	2685
Removal (%)	39.1	34.8

During evaporation, the same 6% decrease in color was observed as in Experiment #1 (Table 15). As for crystallization, the color and polysaccharide results were in the same range as previously. The color occlusion in the crystal was also the same: 9%. Polysaccharide occlusion was a little higher with 19% instead of 14% as in the first experiment (Table 15).

Table 15. Color and polysaccharide removal through process.

Samples	Color (Icu)	Removal	Polysaccharides (ppm)	Removal
Clarified Juice	11 365 ^(*)	-	4129 ^(*)	-
UF Perm + H ₂ O ₂	6629 ^(*)	42 %	2592 ^(*)	37.2 %
Syrup	6215	6.2 %	2687	0 %
Raw Sugar	564	91 %	517	80.7 %

^(*): average values of 3 batches.

The crystal size (250-300 μm) obtained was larger than the ones in Experiment #1 (200 μm max), which may perhaps explain the higher occlusion of polysaccharides in the crystal. Furthermore, the amount of conglomerates is largely decreased compared to Experiment #1.

Another experiment performed with H₂O₂ treated syrup (500 ppm, w/v) produced a sugar with slightly smaller crystals and a little more conglomerates. Table 16 shows the impact of ultrafiltration on the crystallization yield. A decrease of 5-6% in color and polysaccharides removal is noted.

Table 16. Color and polysaccharides removal through crystallization.

Samples	Color (Icu)	Polysaccharides (ppm)
Syrup + H ₂ O ₂	9628	4472
Sugar	1521	1125
Removal	84 %	74.8 %

Summary

The quality of the sugars produced in both experiments was compared to the seasonal average of A sugars from two Louisiana mills from two different grinding seasons, shown in Table 17. It is clear that the UF + H₂O₂ treated sugars produced a higher quality sugar than the mill sugars on either color and polysaccharide content, with quality gain average values, respectively, of 45.2 % and 55%.

Table 17. Improvement in the quality of sugar using ultrafiltration and hydrogen peroxide.

	Color		Polysaccharide (ppm)	
	Sugar 1	Sugar 2	Sugar 1	Sugar 2
UF + H ₂ O ₂ sugar	626	564	303	517
Mill # 1 sugar (Season avg)	1006		686	
Quality gain	37.8 %	43.9 %	55.8 %	24.6 %
Mill # 2 sugar (Season avg)	1179		1354	
Quality gain	46.9 %	52.2 %	77.6 %	61.8 %

The new boiling pan, apart from being much easier to manipulate, allows to treat a higher quantity of product and to produce larger sugar crystals with the same occlusion rate of color and polysaccharides as formerly. However, in order to get closer to industrial crystal size, the boiling needs to last longer. The limitation here is the low power of the stirring system that doesn't match the high viscosity of the massecuite, which will be addressed in future experiments.

EFFECT OF HYDROGEN PEROXIDE ON BEET THICK JUICE

The purpose of this experiment was simply to test the effect of hydrogen peroxide on the color of some highly colored beet thick juice. Future studies may address improvements provided by hydrogen peroxide when difficult thick juices arise due to deterioration of beets in the pile, frozen beets, or other unusual circumstances.

Procedure

Hydrogen peroxide was injected at 500 and 1000 ppm (w/v) into hot thick juice (80 to 95° C). Two different beet thick juices were treated: Tank 7, labeled "hard to filter" and Tank 10B labeled "high color" by the factory that supplied the samples.

Results

Addition of hydrogen peroxide immediately caused production of a great amount of foam. This was correlated to the temperature of the juice -- the higher the temperature, the greater the quantity of foam. We also noted the apparent loss of sucrose (0.56 to 2.36% drop), and the appearance in the juice of an extra peak coinciding with the retention time of raffinose. There was no concomitant increase in the invert level. Table 18 shows the effect of hydrogen peroxide on the color. Color decrease was 38-48% at the 1000 ppm level of H₂O₂ and 19-30% for the 500 ppm level.

Table 18. Hydrogen peroxide effect on color of beet thick juice

	Tk10B			Tk7		
	Original color	500 ppm	1000 ppm	Original color	500 ppm	1000 ppm
	15,724	12,739	8063	8824	6103	5420
% Removed	---	18.9	48.7	---	30.8	38.6

SUMMARY

These experiments have shown that hydrogen peroxide is a potential aid in the processing of sugar cane and sugar beet juice. Combining hydrogen peroxide with membrane filtration holds promise in producing high quality, low color raw sugars and edible sugars from the factory. Hydrogen peroxide may also help to ameliorate problems in the factory when very high color juices are a problem.

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ASSESSMENT OF MOLASSES EXHAUSTION IN LOUISIANA MILLS

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ABSTRACT

Weekly molasses samples from the Louisiana mills for the 2000/01 and the 2001/02 seasons have been analyzed to assess the level of exhaustion of final molasses. Laboratory exhaustion tests support the use of the South African target purity equation, which is considered to be the most appropriate as a benchmark. Relationships have been developed to enable refractometer brix and conductivity ash to be used as reliable substitutes for true solids and sulfated ash. In addition, the two season's samples have been subjected to NIR analysis. Calibrations are excellent, enabling NIR to be used in the future for rapid and reliable assessment of molasses exhaustion. The results of the surveys indicate that C massecuite brix and C massecuite purity play an important part in the achievement of a close approach to the target purity in the factories. Significant reductions in molasses purity can be achieved, with substantial savings for the Louisiana industry. The analyses of monosaccharides indicate the occurrence of Maillard reaction in the massecuites, which hampers good molasses exhaustion.

INTRODUCTION

The loss of sugar in molasses is generally the largest loss suffered by a sugar mill. It is therefore important that reliable data on molasses exhaustion be obtained. The normal measurements used in a sugar mill laboratory are not accurate enough at the low purities associated with final molasses. Chromatographic methods of measurement of sugars content in molasses are now well developed and can be relied upon to give accurate and reliable answers.

In the past, Audubon Sugar Institute (ASI) undertook analyses of molasses samples for the mills in Louisiana. This was discontinued after the 1997 season, but it was considered to be important to reintroduce the analyses of weekly composite molasses samples to provide the mills with reliable information on how well the molasses is being exhausted. This has been done for the past two seasons.

Since the analyses are time consuming, simplifications involving the use of RDS (brix by refractometer) instead of total solids and conductivity ash instead of sulfated ash have been investigated. In addition the analyses have been done in parallel using NIR spectroscopy, with the hope that analyses can be done reliably and more quickly using this technique.

In order to assess the degree of exhaustion achieved, a benchmark is necessary. This is generally available in the form of a "target purity" equation. A number of these have been proposed in the past, and it is necessary to choose the most appropriate for Louisiana conditions.

Background to Target Purity Equations

A number of different target purity equations have been proposed over the years. Essentially they have been derived through laboratory trials on various molasses samples under controlled conditions, thus establishing in essence equilibrium purity under specified conditions.

A target purity can be thought of in two ways, either the lowest molasses purity achievable in a laboratory (equilibrium purity), or the target realistically achievable in a factory. The former is preferred, since it is an objective standard related to what can be achieved, and is not dependent on a subjective assessment of what is acceptable. The difference between actual and target purities is referred to as the Target Purity Difference (TPD) and gives an indication of potential improvement.

The target purity is significantly affected by the measurement methods used in the process of deriving the formula. The effect of analytical methods makes it essential to specify the methods used for each target purity equation. Those that have been used in the past are shown in Table 1.

Table 1. Target purity equations proposed in the literature.

Reference	Equation	Analytical Method			
		Dry Solids	Sucrose	Monosaccharides	Ash
Foster 1960	$40.7 - 17.8 \log(RS/A)$	Drying	Double pol	L & E	Sulfated
Miller et al 1998	$39.4 - 10.6 \log(RS/A)$	Drying	Double pol	HPLC	Sulfated
Bruijn et al 1972	$39.9 - 19.6 \log(RS/A)$	Drying	L & E	L & E	Sulfated
Rein & Smith 1981	$37.7 - 17.6 \log(RS/A)$	Drying	L & E	L & E	Sulfated
Rein & Smith 1981	$33.9 - 13.4 \log(RS/A)$	Drying	GLC	GLC	Sulfated
Smith 1995	$43.1 - 17.5[1 - \exp(-0.74RS/A)]$	Drying	GLC	GLC	Sulfated
Audubon Sugar Inst 1993	$42.4 - 12.3 \log(RS/A)$	RDS Correlation	HPLC	HPLC	Conduct.

The monosaccharides, fructose and glucose, are most accurately measured by GLC (gas liquid chromatography) or HPLC (high performance liquid chromatography). They are commonly referred to as reducing sugars (RS) and measured by the Lane & Eynon method (L&E). Reducing sugars decrease the solubility of sucrose in molasses, while most inorganic components, which constitute the ash, tend to increase the solubility. These target purity equations all take these two effects into account in the ratio of reducing sugars to ash (RS/A). This has been found to be a convenient approach, leading to a simple form of target purity equation. High values of RS/A will generally enable lower molasses purities to be achieved.

Choice of Target Purity Equation as a Benchmark

The ASI (Audubon Sugar Institute) equation was derived originally simply by fitting the lower range of factory molasses purities and is now considered too lenient (Saska, *et al.*, 1999). The South African formula (Rein and Smith, 1981) was assumed to be the most appropriate for the following reasons:

- It is based on an extensive experimental program
- It makes use of the most accurate and reliable measurements (GLC)
- It has been used extensively in Southern Africa for 20 years, and has been found to be reliable under all conditions
- Subsequent laboratory exhaustion work on molasses from other cane producing areas has confirmed its general applicability, to within 1 unit of purity (Sahadeo, 1998)
- A recent survey of molasses from around world shows TPD values in the range of 3 to 7 units, i.e. in the expected range, confirming its general applicability (Sahadeo, 1999)

The use of HPLC techniques has now been shown to give accuracy similar to the use of GLC, on which the equation was derived. HPLC is a much easier technique to use, and is accepted for use with this equation.

It has been shown that in the Tongaat-Hulett mills in South Africa, the TPD values recorded are very similar to the purity rises measured on curing (Smith, 1995). This indicates that the massecuite is well exhausted after the crystallizers, and provides further evidence for the suitability of this formula.

In practice, therefore, it is very unusual for a factory to achieve a zero TPD. The best factories will report a TPD of around 2 to 3 units, roughly equal to the rise in mother liquor purity across the centrifugals.

PROCEDURE FOR THE ANALYSES OF WEEKLY MOLASSES SAMPLES

Sample handling and preparation

Upon arrival each sample is thoroughly mixed and a portion is separated for analyses. The separated portion and control samples are coded and sent to the laboratory. The original samples are stored for future analyses if required.

When the samples are received in the laboratory, all are diluted approximately 1:1. The diluted samples are then used straight or diluted as required for analysis, for RDS (Brix), pol, conductivity ash, sucrose, fructose and glucose. After analyses the remainder of the diluted sample is transferred to a 50 ml centrifuge tube, frozen and retained. At the end of the season the diluted samples are analyzed by NIR and for dry solids by vacuum oven drying.

Weekly results are reported to each mill by fax and the results are posted on the ASI's website.

Procedures

Pol: Twenty-six grams of the 1:1 diluted sample is weighed in a 200ml volumetric flask and diluted to volume with deionized water. The solution is then transferred to a glass jar and two teaspoons of Octapol are added. The sample is shaken well, filtered and read on the saccharimeter. The temperature of the sample is taken immediately after the reading is obtained on the saccharimeter

Dry Solids: These were done at the end of each season on stored diluted samples, by vacuum oven drying (ICUMSA Method GS4/7-11)

(Brix): The diluted sample is read directly on the refractometer. True solids were not measured directly during the season since the analyses are lengthy and would delay getting results back to the mill. During the 2000 season, True Solids were calculated from a relationship developed by Matthesius and Mellet (1976):

$$\text{True Solids} = 100 / ((101.3 / \text{Refractometer Brix}) + (0.932 / \text{True Sucrose}))$$

During the 2001 season, True Solids was estimated from a regression analysis of data from the 2000 season, based on 195 samples, with $r^2 = 0.93$:

$$\text{True Solids} = 6.11060 + (0.8419 \times \text{RDS}) + (0.3637 \times \text{Ash})$$

After dry solids were completed on the 2001 season samples, the correlation did not prove to be as good as with the 2000 season. Further statistical evaluation arrived at the following equation, using pooled data from both seasons (430 data points, $r^2 = 0.85$):

$$\text{True Solids} = 0.5345 + (0.9519 \times \text{RDS}) + (0.1460 \times \text{Ash}) + (0.0347 \times (\text{F} + \text{G}))$$

Conductivity Ash: In 2000, the ASI procedure was used. A one percent molasses sample is prepared by diluting four grams of 1:1 sample solution to 200 ml in a volumetric flask. The sample is then brought to 20°C in a water bath and the conductivity is read in mS on a conductivity meter. The ash is calculated using the following formula:

$$\text{Conductivity ash} = 9.224 \times K \times C + 2.5119$$

K = cell constant ($.99 \text{ cm}^{-1}$).

C = measured conductivity in mS

This formula was derived from data collected over three (1990-1993) seasons on 405 samples.

In the 2001 season, the ICUMSA Method GS1/3/4/7/8-13 for sulfated ash estimation using conductivity of a diluted solution was adopted. The main reason for the change is the fact that the ASI method does not account for sample amount or dry solids of the sample. The ICUMSA method uses the following formula:

$$\text{Conductivity ash} = (16.2 + (0.36 \times D)) \times (C - C_w) \times (5/S)/10000$$

$$D = \text{g RDS}/100\text{ml}$$

$$C = \text{conductivity of sample in } \mu\text{S}/\text{cm}$$

$$C_w = \text{conductivity of water in } \mu\text{S}/\text{cm}$$

$$S = \text{g sample}/100\text{ml}$$

Sugars by HPLC: Approximately one gram of the diluted sample is weighed into a 100ml volumetric flask. The sample weight is recorded to a minimum of three decimal places. The sample is then diluted with Type I water and filtered through a 0.45 μm filter into a sample vial. The sample vial is placed into the refrigerated auto sampler and the true solids weight, calculated from the Brix reading of the 1:1 solution, is entered into the integrator along with the sample number. Standards are run at the beginning and end of each sample set and every five to six samples to verify accuracy (ICUMSA Method GS7-23).

HPLC analyses for the 2001 season were completed using the same equipment except the integrator was replaced with a computer and Dionex Peaknet 5.01 software.

Equipment used for analyses

Apparent Purity:

Bellingham and Stanley Limited RFM340 refractometer

Rudolph Research Autopol IIS Saccharimeter

Conductivity Ash:

Fisher Scientific Accumet Basic AD30 conductivity meter

Sugars by HPLC:

Column: Bio-Rad HPX-87K 300 X 7.8mm

Column Heater: Waters Column Heater Module at 85°C

Solvent: 0.01 M K_2SO_4 at 0.6 ml/min

Detector: Waters 410 Differential Refractometer

Integration: PC with Dionex Peaknet 5.01 and UI20 universal interface

Auto Sampler: Bio-Rad AS100 HRLC with 20 μl sample loop

Pump: Waters Model 510

Estimation of Dry Solids from RDS

After the 2000 season, one hundred and ninety five of the samples were analyzed by vacuum oven drying to determine total dry solids (DS). During the 2000 season, the formula for calculating dry solids, which was designated as True Solids (TS), used RDS and sucrose. Since RDS and sucrose are equal in a pure sucrose solution and the True Solids did not correlate to the DS, other constituents were evaluated.

Statistical analysis of the data using SAS/STAT software indicated that ash content was significant in calculating TS. The following formula was derived and used for the 2001 season:

$$\text{True Solids} = 6.11060 + (0.8419 \times \text{RDS}) + (0.3637 \times \text{Ash})$$

After dry solids were completed on the 2001 season samples, the correlation did not prove to be as good as with the 2000 season. Further statistical evaluation determined that the reducing sugars were also a significant factor. The reducing sugars were higher for the 2001 season, while the ash was lower. This is why the reducing sugars are statistically significant in 2001. This also indicates that formulas such as this and calibrations of instruments like the NIR will require several years to develop properly. The following equation was derived from the combined data from 2000 and 2001:

$$\text{True Solids} = 0.5345 + (0.9519 \times \text{RDS}) + (0.1461 \times \text{Ash}) + (0.0347 \times (\text{F}+\text{G}))$$

This equation will be used for the next season. Dry solids will be run at end of next season to add to the data set.

Laboratory Exhaustion Trials

A previous lab exhaustion trial gave results that supported the applicability of the target purity equation being used (Saska, *et al.*, 1999). With the apparent exhaustion problems at Factory Q, samples of molasses from that factory were collected and subjected to exhaustion trials at ASI. The procedures and the equipment used are described elsewhere (Saska, *et al.*, 1999). The molasses was concentrated in a 150 L pilot pan, seeded with fine sugar, further concentrated to the required consistency, and cooled down to 40° C in pilot crystallizers. Samples were taken periodically for analysis of mother liquor purity. The results as a function of cooling time are shown in Figure 1.

Two sets of runs were undertaken, with a high and a low brix after concentration being used in each set of runs. The two lower curves in Figure 1 represent the high brix case, and show that after about 50 hours very little extra exhaustion is achieved. The target purity predicted by the equation for this massecuite is 36.0, and it can be seen that this purity is achieved.

Figure 2 shows the final purities achieved as a function of mother liquor brix. This emphasizes the importance of concentrating up the massecuite to as high a level as possible in order to achieve good exhaustion. The final purities are also shown as a function of Impurity / Water ratio (I/W) in Figure 3. It is apparent that the target purity is achieved at a value of I/W = 4, which

agrees well with values obtained in the work done to derive the target formula (Rein and Smith, 1981) and values given by Miller, *et al.* (1998).

SEASON SURVEY RESULTS, 2000/01 AND 2001/02

The average values of molasses true purity are shown for the two seasons for all Louisiana mills in Figure 4. Note that some mills only provided a few samples during the 2000 season, in particular Factory J. However, by the time the 2001 started, all mills submitted samples from the beginning of the season. Values of the TPD for the mills are shown in Figure 5. Season average values for all mills are shown in Figure 6, and the variation during the season of the average monosaccharide / ash ratio is shown in Figure 7.

Although the molasses purities in the 2001 season appeared to be a bit lower in Figure 4, inspection of Figure 7 shows that this was due to a higher (F+G)/ash ratio in 2001. The TPD values are no better in 2001 than in 2000. The monosaccharide / ash ratio shows very different behavior in the two seasons, reflecting different climatic and growth conditions for the cane crop in the two years. The TPD values in Figure 5 show higher values at the beginning and end of the season, reflecting startup and liquidation problems.

Figure 8 shows how the pol/sucrose ratio in molasses varied during the two seasons. This illustrates the unsuitability of the pol measurement for accurate molasses sugar analysis. Note that the cause of the dramatic shift in pol/sucrose ratio in the middle of the 2000 season is not evident in TPD values based on true sucrose measurements.

Of interest is the performance of one of the mills, Factory Q, which is generally known as an efficient processor. A graph of its TPD in 2000 is shown in Figure 9. Initially it showed a very high TPD value of around 12 units; midway through the season it made some substantial improvements and the TPD dropped down to a respectable number of around 5 units.

Effect of Factory Operating Conditions on Molasses Exhaustion

Average weekly figures for C massecuite brix and purity were obtained for each mill for the 2001 season to assess how these operating parameters affect TPD values. Attempts to pool the data for all mills were hampered by the fact that 6 of the 16 mills still use spindle brix, and one mill changed from spindle to refractometer during the season. Thus data for the mills were studied individually. In nearly all cases, the data when plotted showed that lower values of TPD were obtained when the C massecuite brix was higher and when the C massecuite purity was lower. In spite of the low number of degrees of freedom (about 12 data points for most mills), statistically significant correlations via linear regression analysis were evident at 7 of the 17 mills. Examples of the correlations for two of the mills (factories K and W) are shown in Figures 10 to 13. The best correlation was achieved at factory W because of the wide range in brix and purity; as would be expected, where the range was small for a mill it was more difficult to find significant correlations.

It is significant that these correlations are evident in spite of all the other factors that can affect molasses purities in practice. High massecuite brix is important as water is the most melassigenic

component and a high I/W ratio must be achieved if a low TPD is sought. It is estimated that a refractometer massecuite brix at strike of at least 97 should be achieved if good molasses exhaustion is to be obtained.

The C massecuite purity has a direct bearing on the quantity of C massecuite to be handled. It can be shown by calculation that increasing the C massecuite apparent purity from 53 to 61 increases the quantity of C massecuite to be processed by 55%. An increase from 53 to 57 massecuite purity increases the quantity of C massecuite to be handled by 21%. The C massecuite purity does not affect the combined quantity of A and B massecuite, whether bagging A and B sugars or whether using a double magma system. It is desirable therefore to attempt to achieve a 53 C massecuite apparent purity.

Use of NIR for Molasses Analysis

All NIR analyses were conducted using a Foss 5500 spectrophotometer with a beverage analyzer attachment. The 1:1 dilutions were read directly on the beverage analyzer.

Data from two seasons from all mills in Louisiana has been used for NIR analysis and calibration. Calibrations for the individual seasons and for the two seasons combined have been generated. Table 2 gives the R^2 and SEC values for each calibration.

Table 2. Correlation information from NIR calibrations.

Constituents	2000 Season			2001 Season			Combined		
	SEC	n	R^2	SEC	n	R^2	SEC	n	R^2
RDS	0.12	189	0.99	0.10	226	0.99	0.12	416	0.99
Pol	0.25	190	0.93	0.31	223	0.95	0.28	409	0.95
Sucrose	0.27	190	0.89	0.22	224	0.97	0.24	414	0.95
Glucose	0.13	189	0.93	0.15	225	0.97	0.11	412	0.98
Fructose	0.10	189	0.92	0.12	229	0.97	0.12	415	0.97
Ash	0.15	192	0.95	0.11	226	0.97	0.13	421	0.97
DS	0.16	187	0.97	0.27	230	0.93	0.24	413	0.95

The combined calibration will be used for the 2002 season. The Foss WinISI software has the capability to determine if a sample spectrum is an outlier. Any outliers and validation samples will be analyzed to update and verify the calibration.

DISCUSSION

The approximation for total solids obtained via the correlation equation is considered to introduce a negligibly small error. Indeed it is considered that the accuracy of the whole determination is only within about one unit. Similarly it is considered that the use of conductivity ash is adequate for purposes of determining target purity.

It appears that the use of NIR analyses will be adequate for determining molasses exhaustion in future. The correlations are very good and the standard errors on all constituents are satisfactorily low.

Dextran affects the viscosity of massecuites, and above levels of about 10,000 ppm on brix (roughly twice the normal Louisiana level), the target purity should be increased by 1 unit or more (Sahadeo, 1998). In times of long delays, when dextran levels rise, TPD values are expected to be higher.

Apart from the effect of dextran, more and more evidence accumulates to support the use of the South African target purity formula as one that has broad applicability throughout the cane sugar producing areas of the world, either the early equation derived by Rein and Smith (1981) or the later modification suggested by Smith (1995) to represent better the low monosaccharides/ash range. In many cases direct comparison of results between industries is not possible because of different methods of analysis of sugars. It is recommended that HPLC be adopted for the measurement of sucrose as well as monosaccharides, and that the South African target purity formula be tested as a universal target purity formula.

The value of the fructose/glucose ratio (F/G) is of considerable interest. The F/G ratio was measured at 1.0 or just above in mixed juice on some samples from Factory H, but averaged 1.6 for the industry in molasses, with a range of values 1.4 to 2.0. The variation in these values is shown in Figure 14. In general the value in juice should be close to one, and it is the occurrence of Maillard reaction that causes this ratio to change. The South African values in 1999 were 1.2 - 2.0 with an average of 1.4 (Lionnet, 2000). The higher the value of F/G, the greater has been the extent of the reaction.

Maillard reaction is a reaction between reducing sugars and amino nitrogen. It results in the formation of color and aerates the massecuite, significantly increasing viscosity (Newell, 1979). It is also known as the phenomenon that causes molasses swelling in crystallizers and explosions in molasses tanks, since it is exothermic (Wong, *et al.*, 1996). It destroys reducing sugars, forming additional impurity, and increasing molasses losses. Glucose is consumed preferentially in the reaction, which leads to values of F/G greater than one, although some fructose also reacts. The RS/ash ratio is reduced, affecting massecuite exhaustion. High concentrations and high temperatures promote the reaction, with a 5° C increase in temperature leading to a doubling of the reaction rate. The reaction can be minimized by boiling pans at as low a temperature as possible. Particularly with C massecuites, the reaction temperatures should be kept below 63° C (145° F) at strike, and cooled as quickly as possible. Molasses backblending may also help.

CONCLUSIONS

The new target purity formula seems to represent Louisiana conditions, and could have wider applicability in other sugar producing regions.

This work has shown the suitability of NIR for molasses analysis and holds great promise for providing quick and reliable data to the mills. Work will be continued next season to analyze

composite weekly mixed juice samples from each mill in Louisiana, and assess how well NIR can be applied to juice samples. The application in a mill laboratory for routine analyses is not considered to be too far off.

Molasses purities on average are 6 to 8 units higher than they could be in Louisiana. This is worth about 3 units of overall recovery, or \$20M / year to the Louisiana industry. Efforts to reduce molasses purities would be assisted by reducing the prevalence of Maillard reactions. Good exhaustion can be expected if C massecuite refractometer brix can be increased to at least 97 and C massecuite purities lowered to around 53.

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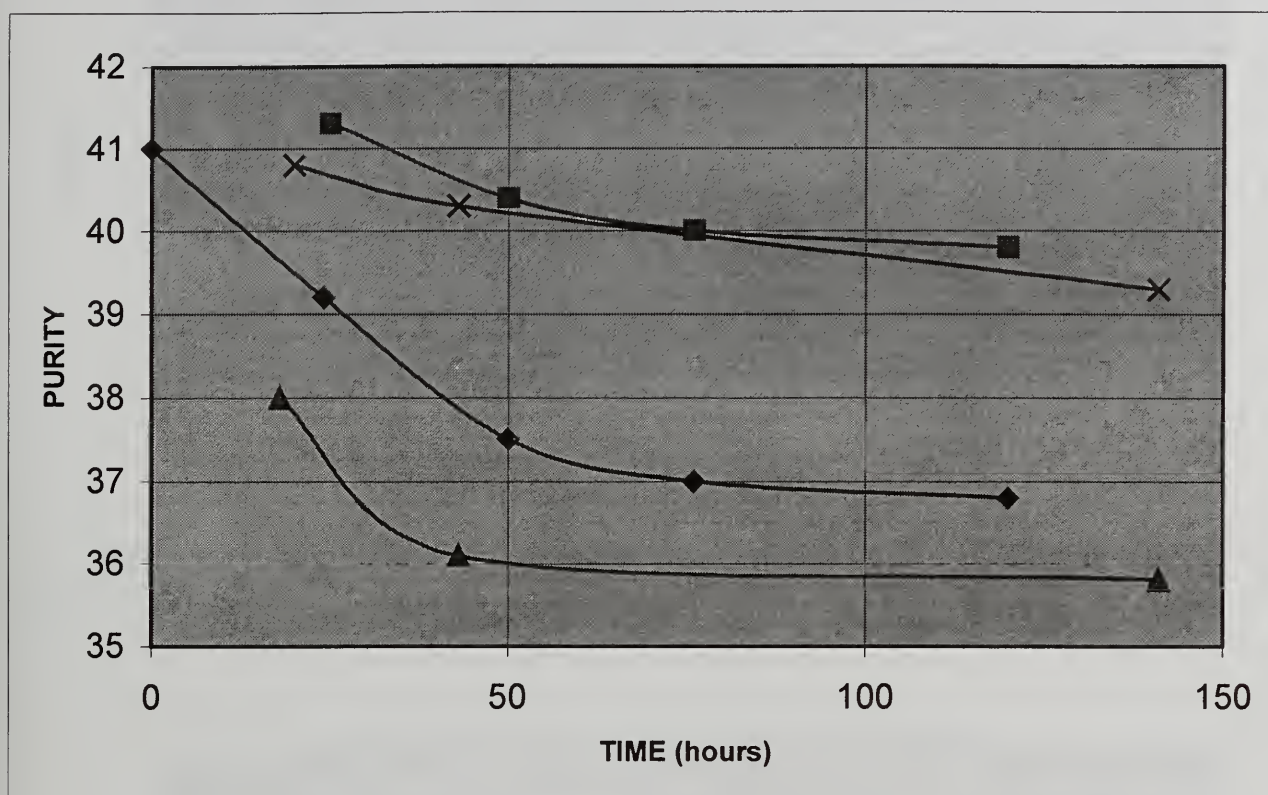


Figure 1. Molasses purities as a function of cooling time in a laboratory crystallizer.

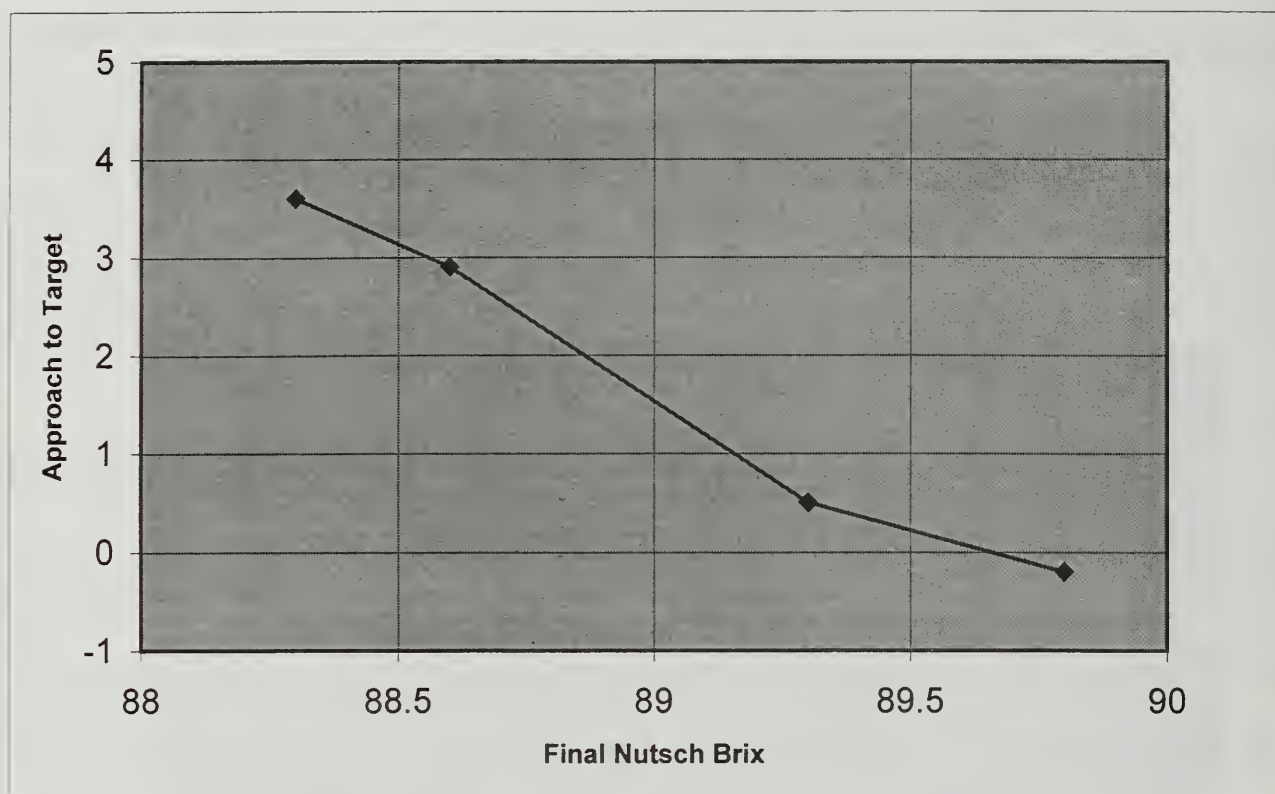


Figure 2. Target purity difference in laboratory crystallization as a function of mother liquor RDS.

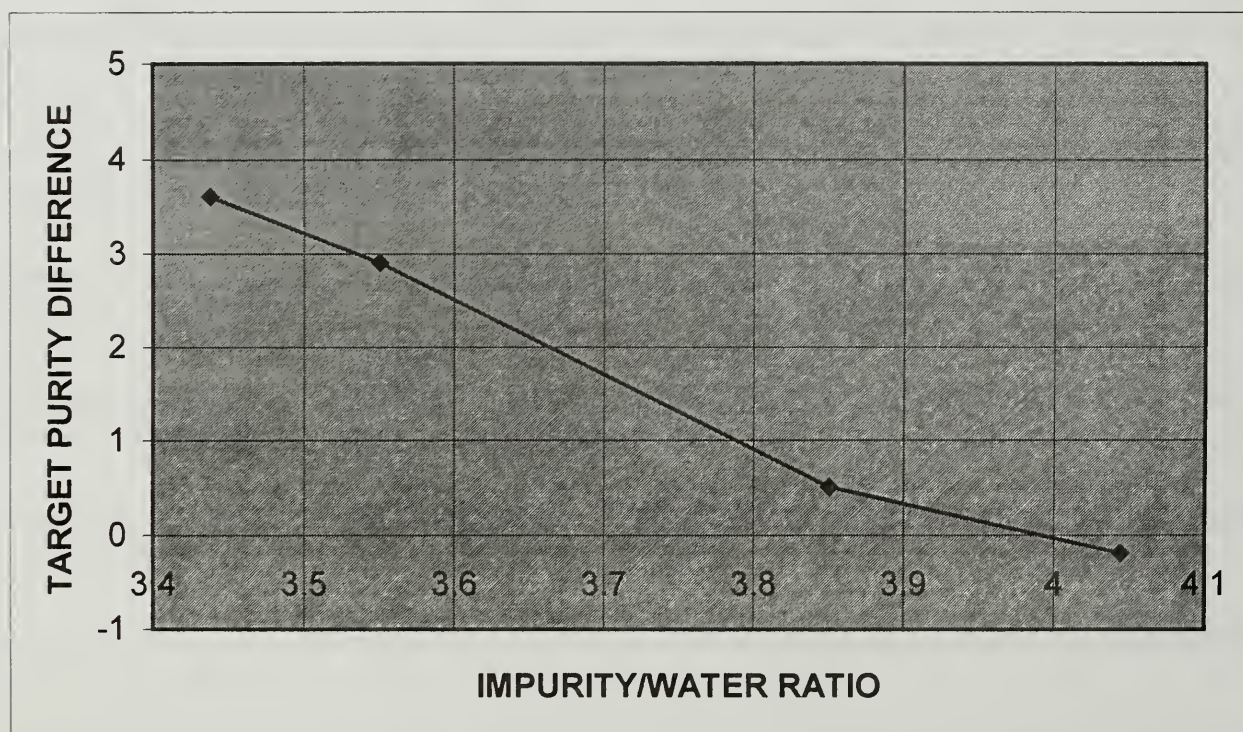


Figure 3. Approach to target purity as a function of Impurity/Water ratio in laboratory crystallizer.

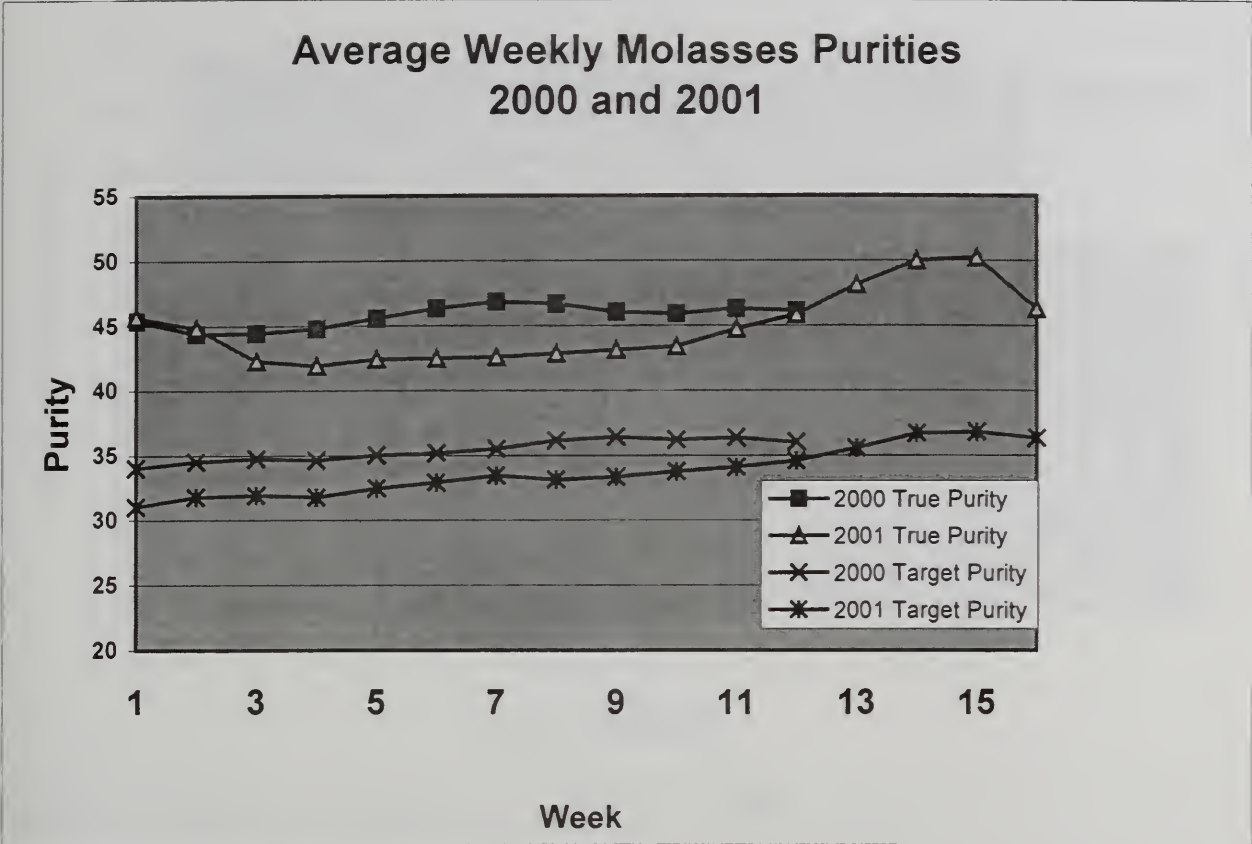


Figure 4. Average target and true purities.

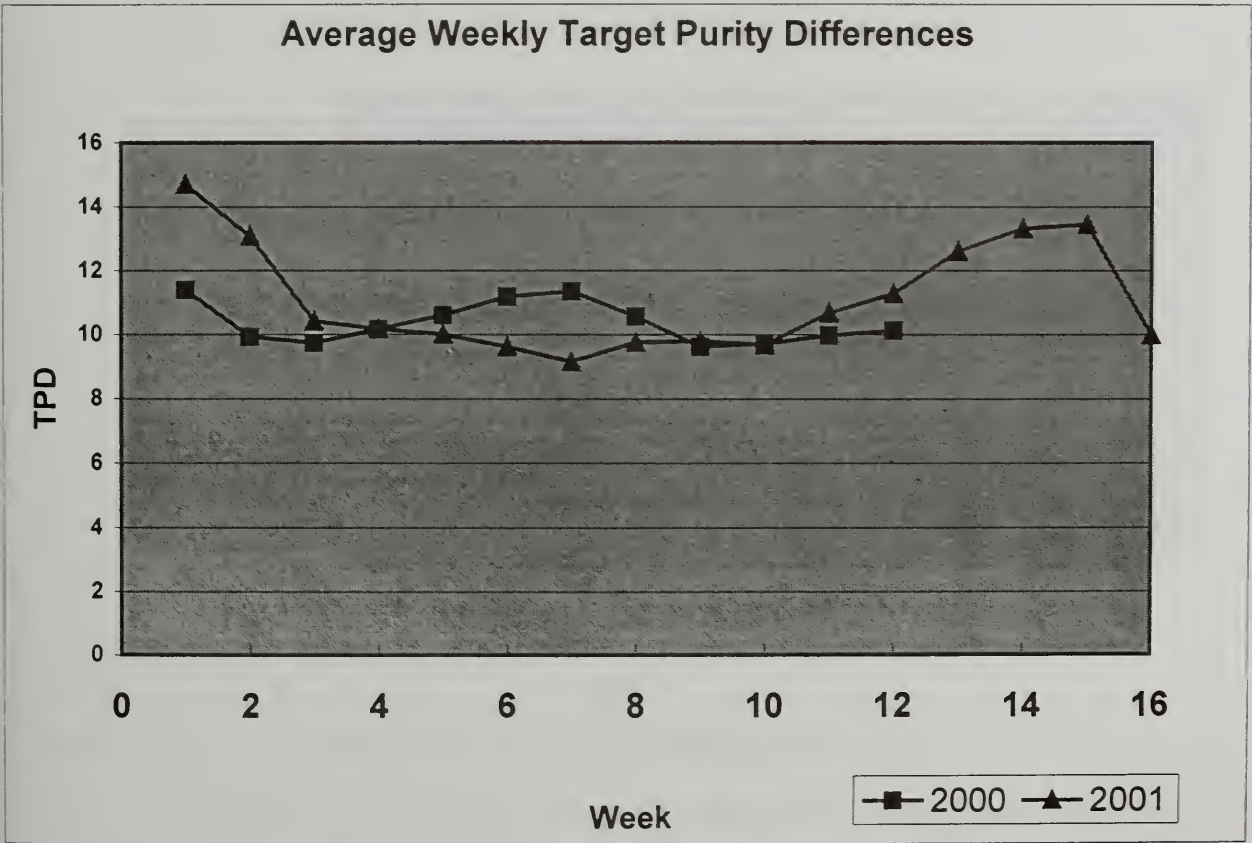


Figure 5. Average weekly target purity differences.

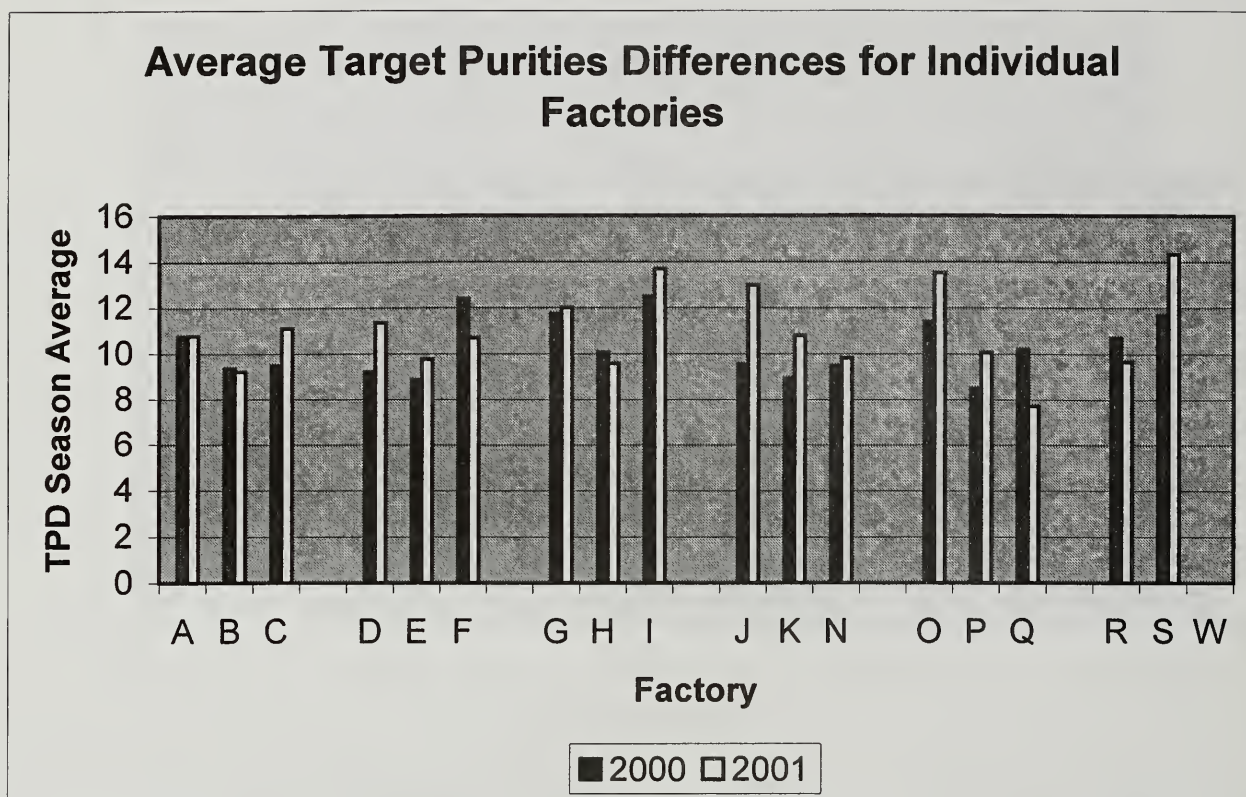


Figure 6. Average seasonal target purity differences.

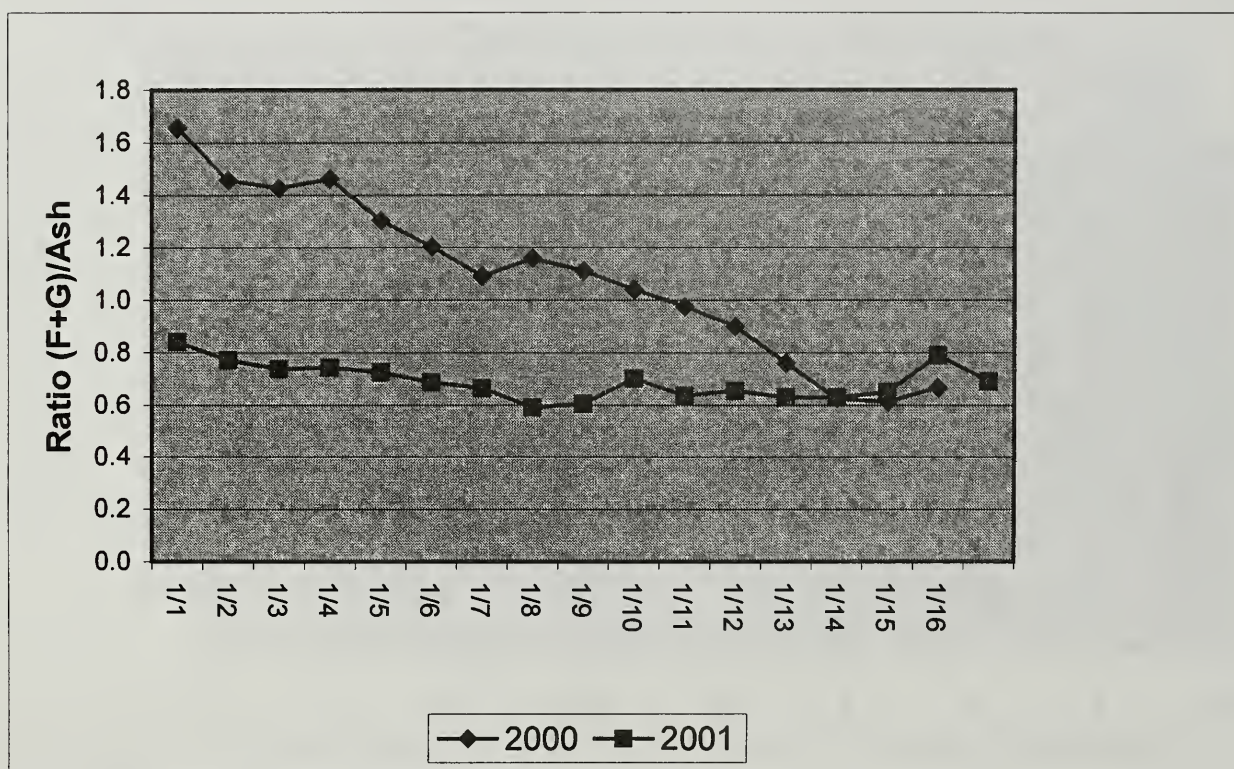


Figure 7. Variation in monosaccharide/ash ratio during the 2000 and 2001 seasons.

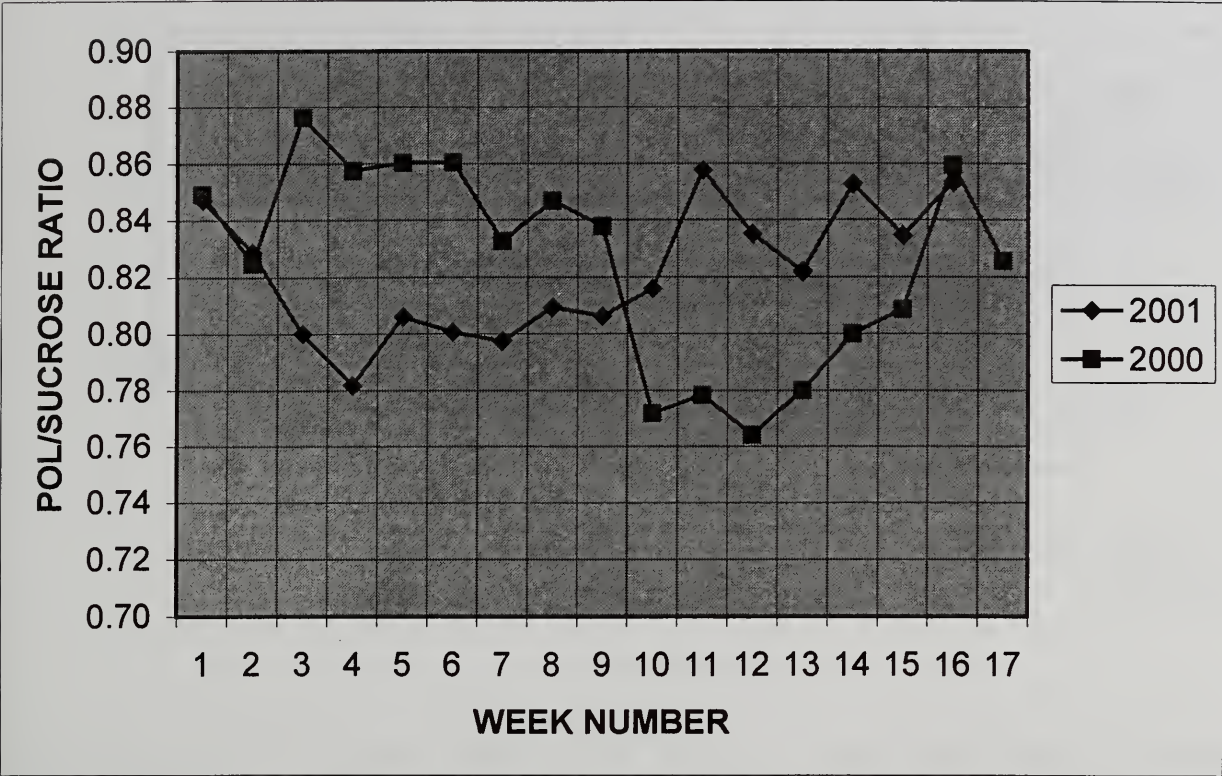


Figure 8. Variation in pol/sucrose ratio in 2000 and 2001 seasons.

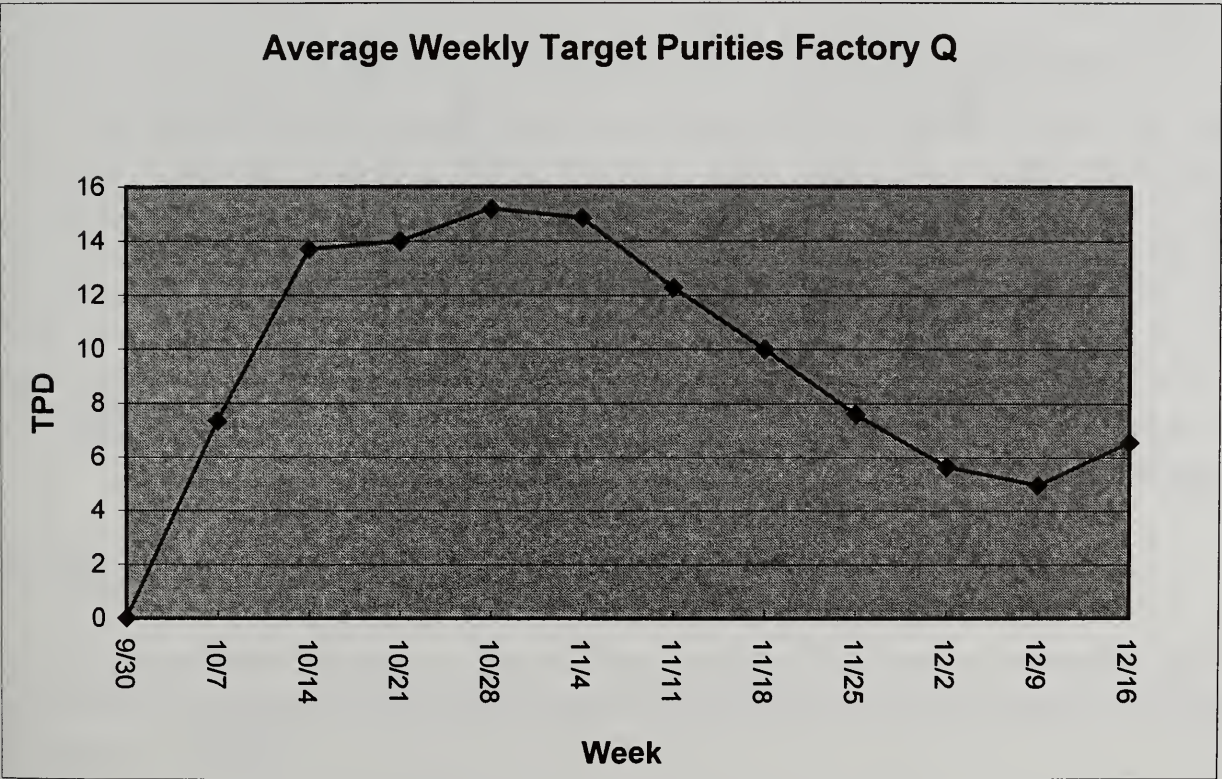


Figure 9. Weekly target purity differences for factory Q.

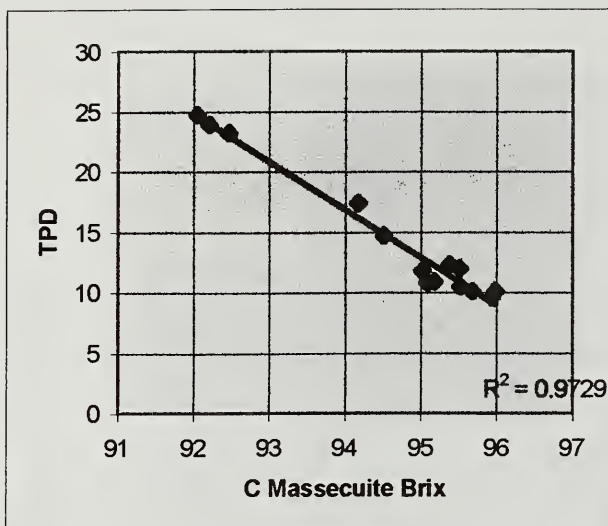


Figure 10. Factory W: TPD vs C-massecuite Brix.

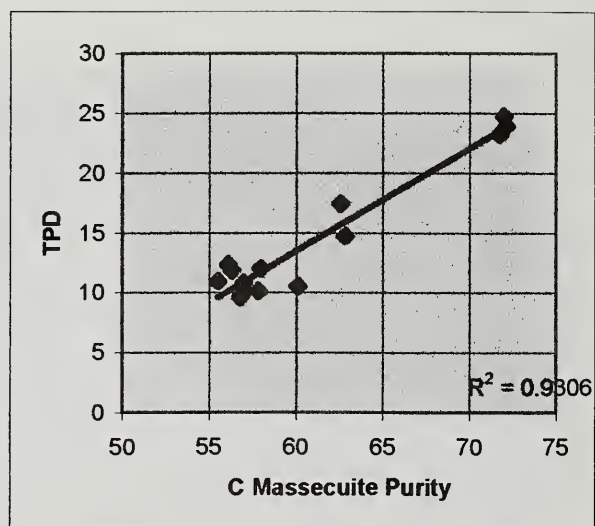


Figure 11. Factory W: TPD vs C-massecuite purity.

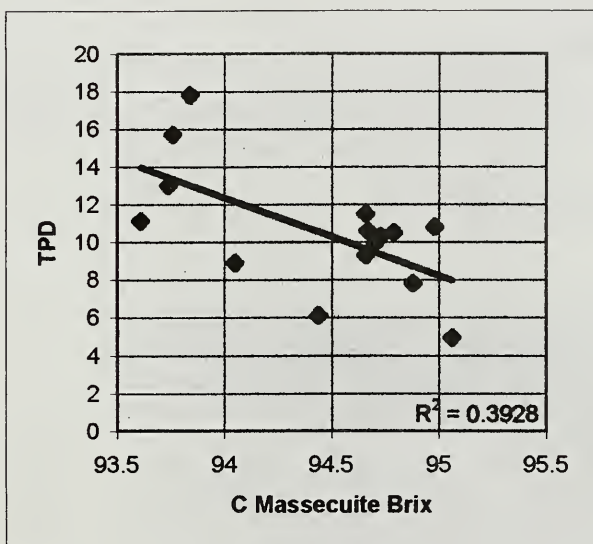


Figure 12. Factory K: TPD vs C-massecuite Brix.

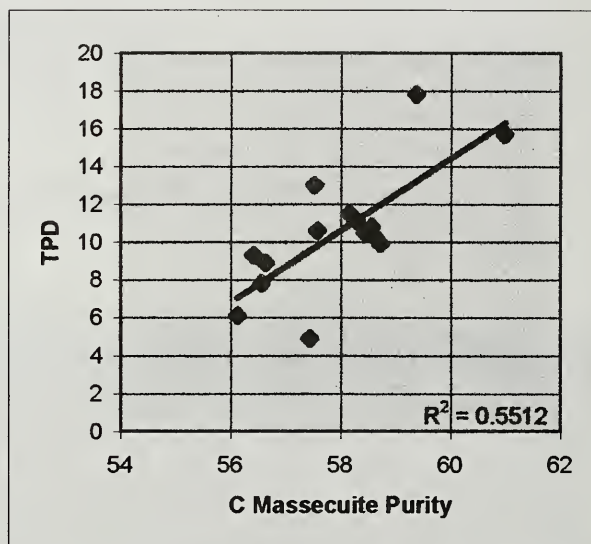


Figure 13. Factory K: TPD vs C-massecuite purity.

POSTHARVEST SUGAR LOSSES IN SUGAR CANE: VARIETAL DIFFERENCES AND EFFECT OF HARVESTING METHOD

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ABSTRACT

Deterioration of the cane and sucrose losses between cutting and milling begin after cutting, increasing with the time the cane remains in the field or in the mill yards. The deterioration rate depends upon the environmental conditions, the cane variety and the management of the harvesting system. In addition, it has been established that leaves and trash also contribute to increased sucrose losses. Sucrose losses of chopped and whole-stalk cane were studied, using burned and nonburned cane of the commercial variety MZC 74-275. The sucrose lost per hour in the field ranged from 0.06% - 0.15% for burned and nonburned whole stalks of all varieties. The rate of deterioration was higher for chopped sugarcane (0.15%-0.25% per h). Sugar losses from 5-16% per day were observed during the time whole stalks were stored in the mill yards. Statistical analyses showed that for 1% of trash in clean cane, there is an average sucrose loss of 2.0 kg/t of cane. Results of research conducted in Colombia indicated that mechanical harvesting of nonburned cane increased trash in cane delivered to the factory, resulting in lower sugar yields. The establishment of reliable methodologies based on HPLC and NIR for estimating sugar losses and trash in commercial cane provided useful information for investment decisions to increase overall sugar yield in some sugar mills.

INTRODUCTION

In recent years the Colombian sugar industry has been interested in evaluating the effect of sucrose losses attributed to burning practices and the time the cane is left in the field or mill yards prior to milling.

Significant differences can occur in the losses of sugar when different harvesting systems are used simultaneously by different producers or sugar mills. An immediate effect of the cane harvest, especially of mechanical harvesting is the incorporation of a large amount of trash and greater exposure to microbial attack by bacteria such as *leuconostoc*, which reduce factory yield

as has been reported by different authors in Australia, Louisiana (USA) and South Africa (Egan and Rehbein, 1963; Irvine and Legendre, 1973; Wood, 1973). Similarly, recent studies in Colombia (Larrahondo, *et al.*, 1999; Osorio, *et al.*, 1997), using periodic samples of juices and HPLC (high performance liquid chromatography) analyses made it possible to establish levels of sucrose losses (sucrose % cane), ranging from 0.2-6.0% at 48 h after burning and cutting whole cane (manual harvesting).

On the other hand, CENICAÑA conducted a study to establish a series of mathematical equations based on sucrose losses for different varieties and different levels of trash in order to provide guidelines that would reduce the sucrose losses between the cutting and the milling.

MATERIALS AND METHODS

Sucrose losses after burning and cutting cane First trials of cane left piled in the field

Given that the variability in the weight, length and sucrose content of the stalks sampled has been an obstacle for the precise determination of sucrose losses after harvesting, a strategy of “nondestructive” sampling of the stalks was designed, based on taking small amounts of juice periodically, extracted using a sharp pointed instrument in three different parts of stalks previously marked before the harvest and piled after the burning–cutting under commercial conditions in the field for 120 h. The samples of juice extracted from five stalks (approx. 6 ml) were analyzed via HPLC, using a minimum of three replications during the trial period. The sampling and evaluations of sucrose were done before and after the burning and every 24 h, based on the time of the cut. Similarly, the stalk samples were weighed to observe the weight losses. In these first trials of piling the cane in the field (four), uniform lots of 22.7 ha of commercial cane of the var. MZC 74-275 with ages ranging from 12-13 months were selected.

Sucrose losses of the cane stacked in wagons

The changes in the concentration of sucrose of variety MZC 74-275 (plant crop), stored after the harvest in wagons, were evaluated over a period of 136 h. The variety was harvested at the age of 12.3 months using both the manual (whole stalks) and mechanized systems (average pieces of 30 cm) and under conditions of burning and nonburning. The previously described system of nondestructive sampling was adopted; and the samples of juices (approx. 6 ml) extracted from five stalks (previously marked) were analyzed via NIR (near infrared spectroscopy), using a minimum of three replications for diagnosing sucrose losses throughout the trial period. The NIR calibrations and validations were based on analyses of primary juices using HPLC.

Sucrose losses due to the time the cane remains in mill yards

Occasionally large piles of cane can be seen in the Colombian mill yards, especially on weekends or when milling is suspended. Thus it was decided to monitor sucrose losses resulting from cane kept in the mill yards. Var. MZC 74-275 and CC 85-92, cut manually after burning or nonburning, was studied. From 1000-2000 t of cane per variety (age ranging from 12-14 months) were piled under commercial conditions and left for 24 h. Part of that cane was fed to the

conductor and the mills, every 6 h, based on the piles in the yards. Samples of juice were taken and analyzed in the lab to determine the percentages of sucrose, purity, reducing sugars and dextrins.

Effects of the trash and commercial determination of sucrose losses with different varieties and cutting systems

Based on the commercial information of different varieties (MZC 74-275, CC 85-92, PR 61-632 and V 71-51) supplied by different mills, a multiple correlation analysis of the pol % cane or recoverable sugar was estimated as a function of the trash level and time until processing in order to establish a mathematical equation that would estimate the impact of these two factors on sucrose losses under various harvesting conditions. Similarly, a commercial-scale trial was conducted using var. CC 85-92 (age 13.2 months) to compare the harvesting systems: mechanical cutting/burning, mechanically harvested/green cane, manually harvested/clean green cane, manually harvested/semiclean green cane, manually harvested/burned. For this purpose, about 200 t of cane for each harvesting system was delivered to the factory; and during the milling, samples of prepared cane were taken and analyzed in the lab to determine the levels of sucrose and estimated recoverable sugar (ERS % cane). During the trial, the values of trash and time until processing were recorded for each harvesting system as well as the weight losses of stalk samples.

RESULTS AND DISCUSSION

Sucrose losses after burning and cutting: First trials of piling cane in the field

The methodology of nondestructive sampling of stalks for monitoring and diagnosing sucrose losses for burned cane, cut and stored in the field, made it possible to establish a range of 0.2-6.4% sucrose (sucrose % cane) lost in the first 48 h after cutting, and 3.5-9.0% in 72 h (Figure 1). Additionally the average percentages of sucrose losses based on the time the cane remained in the field, were adjusted statistically according to the following equation:

$$\% \text{ sucrose losses} = 100 - 100 e^{-0.001t}$$

where X = number of h after cutting

$$R^2 = 0.98$$

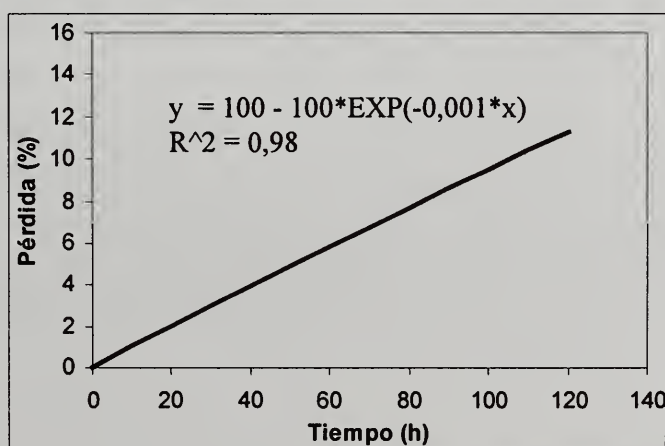


Figure 1. Sugar losses after burning, using nondestructive method and HPLC.

In addition, it was found that the sucrose losses attributable to burning as a harvesting practice, corresponded to an average of 3.0% in a range of 0-6%, determined by the nondestructive method and HPLC.

Sucrose losses in the cane piled in wagons

The concentration of sucrose in the burned and chopped cane (mechanical cutting) decreased by 9% after having being left piled in the wagons for 20 h. The burned and chopped cane had the highest percentages of losses, followed by the chopped green cane. This difference was especially notable in the first 40 h of evaluation. In the whole cane no significant differences were noted between the burned or green cane until after 90 h of being in the wagons (**Table 1**). The results suggest that when programming the harvest, priority should be given to milling the chopped cane, cut mechanically.

Table 1. Percentages of sucrose losses in the cane piled in wagons, after cutting and before milling. Variety MZC 74-275.

Harvesting system		% Sucrose Losses				
		Time (h)	16	40	64	90
Manual cut	Nonburned	0	3.2 ± 3.2	5.6 ± 0.4	8.1 ± 1.4	11.2 ± 0.8
	Burned	0	3.6 ± 2.5	6.6 ± 0.3	8.0 ± 0.1	11.0 ± 0.8
Mechanical cut	Nonburned	0	7.0 ± 2.0	8.0 ± 4.0	8.5 ± 2.1	12.4 ± 2.0
	Burned	0	5.1 ± 4.7	12.0 ± 1.6	13.0 ± 2.1	17.0 ± 5.3

Sucrose losses due to leaving cane piled in mill yards

The general results of this study of piling under the previously described conditions are presented in (**Table 2**). Discounting the effect of the burning on the sucrose losses, the greatest losses occurred from 12-24 h after being left in the mill yards.

With green cane the two varieties, MZC 74-275 and CC 85-92, gave the lowest total losses of sucrose and the best stability for purity. The results also showed that the sucrose losses after 24 h of being left piled in the mill yards ranged from 7.5-11.4%, equivalent to 72 h of storage in the wagons or transportation equipment or 120 h of having been left in the field (6.3-14.3% losses in sucrose % cane). It can be affirmed in general that the sucrose losses in cane after one day in the yards are equivalent to the losses after five days in the field.

Table 2. Determination of sucrose losses and formation of dextran in the cane left piled in the mill yards.

Hours	Var. CC 85-92				Var. MZC 74 -275		
	Burned		Nonburned		Burned	Nonburned	
Dextran (mg/L)	% Losses (Sucrose)	Dextran (mg/L)	% Losses (Sucrose)	Dextran (mg/L)	% Losses (Sucrose)	Dextran (mg/L)	% Losses (Sucrose)
0	0	100	0	106	0	181	0
	112						
6	6.5	317	5.0	119	7.2	184	6.2
	121						
12	7.0	409	6.0	120	9.0	261	10.0
	227						
24	11.4	766	8.5	150	11.4	290	7.5
	230						

Effects of the trash and commercial determination of sucrose losses in different cutting systems

In the lab-scale evaluations conducted at CENICAÑA, it was observed that when trash was added to the samples of clean cane, 1% of plant residue resulted in a decrease of 0.14 units (%) of pol, equivalent to a 1% reduction with respect to the pol % cane present in the clean cane. A slightly higher (0.20 units) reduction for each 1% of mineral matter was observed during evaluations with cane harvested mechanically.

A multiple regression analysis for the commercial data of pol % cane from different varieties, determined at the factory at the moment the cane entered the mills, as a function of the levels of trash and times between the cutting and milling, established that for each percentage point of trash, the pol % cane decreased from 0.15-0.23 units (%), which corresponded to reductions of sucrose (pol % cane) of about 1.0% and 1.5%, respectively (**Table 3**). Similarly, it was possible to determine that although the time between the actual cutting and milling is an important factor, it has less impact on the sucrose losses related to the trash. For each hour the cane remains in the field or in transportation equipment, sucrose losses (pol % cane) ranging from 0.06-0.15% can be observed, which validated the results obtained and reported previously for different laboratory trials with the variety MZC 74-275 (**Table 3**).

Table 3. Commercial validation of the reductions in pol % cane from different varieties, per unit of trash and time the cane remained in the field.

Mill	Reduction pol (%) / 1% trash	Loss pol (%) / h
A	0.23	0.02
B	0.14-0.19	0.01-0.02
C	0.15	0.01
Lab. Trials	0.20	0.012-0.014

The commercial trial of sucrose losses (pol % cane) between the cutting-milling for the mechanical (burned/nonburned) and manual (burned/clean and semiclean green cane) cutting systems established that the greatest losses or reductions (pol % cane) corresponded to the mechanical cutting (range of 18-23%), which had the highest levels of trash and the least retention time (**Table 4**), which suggested that the greatest impact in the losses or decreases in factory yields can be attributed, to a great extent, to the presence of trash incorporated during the harvesting of the cane. On the other hand, the multiple linear correlation analysis among the expected yields (ERS % cane) for each harvesting system, the percentages of trash, and the times between the cutting and milling established that for each unit (%) of trash, the yield was reduced by 0.26 units (%). Similarly, under the conditions of the commercial trial, it was determined that variety CC 85-92 decreased its yield by 0.024 units for each hour the cane remained in the field or in the transportation equipment, according to the following equation:

$$\text{ERS \% cane} = 13.3 - 0.26 (\% \text{ trash}) - 0.024 (\text{time in field})$$

$$R^2 = 0.99$$

Table 4. Sucrose losses (pol % cane), trash content and times between the cutting and milling in different cutting systems.

Cutting System	Trash (%)	Var. CC 85 -92	
		Time (h) between Cutting-Milling	Loss of Sucrose (pol % cane)
Mechanical/ Burned	12.7	17	23.2
Mechanical/ Nonburned	12.8	6.3	18.4
Manual/ Nonburned	2.4	39.0	10.0
Manual/ Burned	0.24	33.0	6.4

Based on the above results and the commercial information of the recoverable sugar (ERS % cane) from different varieties, supplied by some mills, CENICAÑA established mathematical equations (**Table 5**) that made it possible to estimate the impact of trash and time between cutting-milling on the recoverable sugar in the factory (Larrahondo and Briceño, 2001) with acceptable accuracy (close to 95%).

Table 5. Mathematical equations for recoverable sugar (ERS % cane) as a function of levels of trash and time between cutting and milling.

Mill	Equation ¹
A	$12.9 - 0.18 (\% \text{ trash}) - 0.01 (\text{time in field, hours})$
B	$13.3 - 0.19 (\% \text{ trash}) - 0.02 (\text{time in field, hours})$
C	$12.0 - 0.21 (\% \text{ trash}) - 0.01 (\text{time in field, hours})$
Lab Trials	$14.2 - 0.23 (\% \text{ trash}) - 0.02 (\text{time in field, hours})$

$$^1 \text{ ER (\%)} = \text{ERS \% in plant} - F_t (\% \text{ trash}) - F_h (\text{time in field, hours})$$

Where F_t = reduction of ERS % / 1 % trash)
 F_h = Loss of ERS % / h

CONCLUSIONS

- The nondestructive sampling method and the HPLC and NIR techniques have, among their advantages, the guarantee of being able to monitor better and with good precision the sucrose losses between the cutting-milling, using a reduced number of samples.
- The results indicate the importance of minimizing or eliminating the periods of storage of large piles of cane in the mill yards. When it is necessary to pile the cane before the milling it should be left in small piles, either in the field or in the transportation wagons, to prevent significant losses of sucrose.
- The analysis of commercial information made it possible to establish that for each unit (%) of trash, the recoverable sugar is generally reduced from 0.18 - 0.23 units.

- In a commercial evaluation of sucrose losses for different cutting systems, it was found that the greatest losses or reductions in the pol % cane corresponded to mechanical cutting, which had the highest levels of trash.
- The mathematical equations developed integrate loss of recoverable sugar based on trash content and milling delay. This model will be useful in designing programs to improve sucrose recovery for commercial varieties under different harvest practices.

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SOURCES AND INDICATORS OF CANE DETERIORATION

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ABSTRACT

Cane deterioration in the field, factory storage pile, or during factory milling processes, has become a topic of major concern in recent years, particularly in those areas of the world where mechanical harvesting of billeted sugar cane has increased dramatically. Little work has been accomplished on accurately elucidating the relative contributions of microbial, enzymic, and chemical reactions to sucrose losses on cane deterioration at ambient temperatures. This is partly because inaccurate pol measurements have been too frequently used to evaluate sucrose losses. This investigation was undertaken to determine the relative sources of cane sucrose losses by using laboratory tests to compare microbial, enzymic, and chemical deterioration of sucrose in factory cane juice. Heat (boiling temperature) and biocide treated juice, as well as untreated juice, were deteriorated at 27°C (to simulate factory ambient temperature) in an incubator across 71 hours (h). The biocide treated juice retained its dark brown color, fresh odor, initial pH and °Brix levels across 71 h. In strong contrast, after 71 h the untreated juice was light brown, had a strong alcohol odor and markedly lower pH and °Brix levels. The color of the heated juice only reduced after 23 h, and the juice was viscous after 71 h and had neither a fresh nor alcoholic odor. Sucrose, glucose, and fructose were analyzed using gas chromatography. Over the first 14 h of deterioration, 93.0% of sucrose losses were microbial, 5.7% enzymic and 1.3% were chemical (acid degradation). Ion chromatography with integrated pulsed amperometric detection (IC-IPAD) was used to simultaneously analyze for ethanol, mannitol, and oligosaccharides in deteriorated cane juice. The rate of formation of mannitol, produced from the reduction of fructose by mannitol dehydrogenase in dextran forming *Leuconostoc* bacteria, was much higher than associated oligosaccharides or ethanol formation. A further investigation of the use of mannitol as a sensitive indicator of future dextran related processing problems at the cane factory is warranted. Ethanol was shown not to be very useful as an indicator of *Leuconostoc* bacterial cane deterioration.

INTRODUCTION

Cane deterioration in the field, factory storage pile, or during factory milling processes, has become a topic of major concern in recent years, particularly in the U.S. where mechanical harvesting of billeted sugar cane has increased dramatically. Sucrose destruction reactions in cane deterioration include chemical (acid) and enzymic inversion reactions, and those from microbial activity, and can be influenced by cane health and environmental conditions.

Sugar technologists (Morel du Boil, 1995; Lionnet, 1996; Eggleston, *et al.*, 2001a,b) have reported a variety of cane deterioration products to confirm cane deterioration and delay (cut-to-crush time), which have been used to predict and control processing problems at the factory. Such deterioration products have included high invert concentrations, microbial (yeast, bacteria, and fungi) contamination (e.g., ethanol and lactic acid concentrations) and polysaccharides, but not all deterioration products impact future factory processes. Lionnet (1996) stated that a cane deterioration product “will be useful only if it can be related to some aspect of the operations of the factory”. Dextran polysaccharide (formed mainly by *Leuconostoc* bacteria) has often been reported as a cane deterioration indicator, and is responsible for many of the numerous negative impacts that cane deterioration has on factory processing, mostly associated with the rise in viscosity from this polysaccharide. Oligosaccharides are also products of cane deterioration (Morel du Boil, 1995; Ravelo, *et al.*, 1995; Eggleston, *et al.*, 2001b) and are responsible for crystal deformation problems (Morel du Boil, 1991). Ravelo, *et al.*, (1991) reported that the formation of total oligosaccharides was greater than the formation of dextran and ethanol in cane subjected to delays and is, therefore, a more sensitive indicator of cane deterioration.

Little work has been accomplished on accurately elucidating the relative contributions of microbial, enzymic, and chemical reactions to sucrose losses on cane deterioration at factory ambient temperatures. This is partly because inaccurate purity measurements using optical rotation (referred to as “pol” in the sugar industry) have been too frequently used to evaluate sucrose losses (Kulkarni, 2001). The present investigation was undertaken to determine the relative sources of sucrose degradation in cane juice by using laboratory juice deterioration experiments. By using biocide and heat treatments on factory cane juice, the elucidation of sources were made more clear. The investigation was also undertaken to identify deterioration products which can be used as sensitive indicators of deterioration of cane in the field and at the factory, and which could predict future processing problems. Carbohydrates serve as carbon energy sources for many microbes, while sugar alcohols (alditols), alcohols, and organic acids are metabolic products. Ion chromatography with integrated pulsed amperometric detection (IC-IPAD) was used to simultaneously analyze for sugars, alditols, and alcohols in cane juice.

EXPERIMENTAL

Factory Cane Juice Deterioration Experiments. Factory mixed juice (MJ) of pH 5.16, with no preservatives added, was collected from a Louisiana raw sugar factory before screening, and stored in a laboratory -40°C freezer until required. MJ (300 ml) was placed in three separate beakers. In the first beaker, 0.08% sodium azide (0.24g/300 ml juice) was added; in the second beaker, the MJ was brought to a boil and boiled for 1 min, then cooled immediately on ice; in the third beaker, juice was left untreated. The pH of the three samples was adjusted after treatment to the initial pH of 5.16. Adding azide increased the pH to 5.46 so dilute HCl was added to adjust the pH to 5.16. Boiling of the juice reduced the pH to 5.07, and this was raised to pH 5.16 with dilute NaOH. Subsamples (30 ml) from each treatment were then transferred to smaller beakers representing each time of deterioration, and were lightly covered with parafilm and aluminum foil to prevent evaporation, and to simulate juice in closed pipes and tanks in the factory. All beakers were placed in a non-sterile incubator at 27.1°C (approx. ambient temperature in the factory). A beaker representing each of the three treatments was removed after 0, 7, 14, 23, 31, 38, 47, 55, and 71 h and immediately placed on ice. °Brix and pH were measured before storage at -40°C, until further analyses.

IC-IPAD Analysis of Carbohydrates and Sugar Alcohols. Carbohydrates (mainly oligosaccharides from 2 to 12 degrees of polymerization) and alcohols were determined on duplicate juice samples, diluted 1 g/25 ml then filtered through a 0.45 µm filter. Carbohydrate IC-IPAD chromatograms were obtained on a Dionex BioLC instrument. The carbohydrates and alcohols were separated on Dionex CarboPac PA-1 guard and analytical anion exchange columns (250 x 4 mm), at ambient temperature (~25 °C). Flow rate = 1.0 ml/min. Eluent conditions were: 100 mM NaOH isocratic (0.0-1.1 min; inject 1.0 min), a gradient of 0 to 300 mM NaOAc in 100 mM NaOH (1.1-40.0 min), and return to 100 mM NaOH (40.1-45.0 min) to re-equilibrate the column. Oligosaccharides and alcohols (from 100 µl injections) were detected with a PED-2 detector; detector conditions are listed in Eggleston and Clarke (1997). Using a Spectra-Physics SP8880 autoinjector and Dionex Peaknet chromatography software, runs were accumulated of multiple samples and check standards. Oligosaccharides, mannitol, and ethanol were identified by comparing retention times with standards and by spiking with standards. Peak heights were measured to reduce the effect of interfering adjacent peaks. Mannitol, ethanol, palatinose, and leucrose were quantitated in reference to myo-inositol, ethanol, glucose, and sucrose standards, respectively. Other oligosaccharides were quantitated in reference to raffinose.

Sucrose, Glucose and Fructose by Gas Chromatography (GC). The determination of sucrose, fructose and glucose in cane juice by GC was based on the oximation-silylation procedure in ICUMSA GS7/4-22 (1998). See Eggleston et al (2002) for full method.

°Brix. The mean °Brix of triplicate samples was measured using an Index Instruments TCR 15-30 temperature controlled refractometer accurate to ± 0.01 °Brix.

pH. Measurements of pH were at room temperature ($\sim 25^{\circ}\text{C}$), using an IngoldTM combination pH electrode calibrated at room temperature using two different pH buffers (pH 7 and 10). The electrode was connected to a Metrohm 716 DMS pH meter.

Dextran. Duplicate samples were analyzed for dextran using the ASI (Audubon Sugar Institute) II method (Sarker & Day, 1986) which uses dextranase enzymes.

RESULTS AND DISCUSSION

Deterioration of Cane Juices

In order to determine the relative contributions of microbial, enzymic, and chemical deterioration of sucrose in cane juice, laboratory manipulations of cane juice were undertaken. These included the addition of an effective biocide (sodium azide) or heat (at juice boiling temperature). The biocide, sodium azide, was chosen because of its effectiveness to destroy all microbes, which was a requirement of this laboratory study if the relative sources of deterioration were to be quantitated. However, because of its toxic character, it would not be appropriate for use as a biocide in the factory. The pH of the treated juices was adjusted to the initial untreated value, in order to eliminate initial differences caused by acid sucrose inversion.

Initially all samples were dark brown and had the characteristic fresh odor of factory cane juice. The untreated juice began to change to a paler color after 7 h, and by 71 h was a very pale brown and had a “wine” odor. In comparison, the addition of a biocide had a remarkable effect: after 71 h the fresh odor was still apparent and the color unchanged from dark brown. Sodium azide is an effective biocide, which does not allow any microbial growth to occur, therefore, this was the first indication that much of the deterioration was microbial. In further comparison, the heated juice, changed to a pale color only after 23 h, and after 71 h had neither a fresh nor “wine” odor. Moreover, the sample was viscous and gummy in appearance, indicating polysaccharides had formed.

Analysis of Deteriorated Cane Juices

The pH changes with deterioration time are shown in Figure 1. Cane deterioration, as indicated by a reduction in pH, started immediately in the untreated cane juice, with the largest changes occurring in the first 14 h, the rate of pH change decelerating thereafter. The pH also decreased in the heated juice but only after a 14 h delay, indicating either enzymes or microbes (the heat would have denatured the enzymes and destroyed or vastly reduced the numbers of microbes) are responsible for the initial change in pH. In the biocide control sample, there were no marked changes in pH across 71 h, further evidence that microbial growth is mostly responsible for cane deterioration.

Fig. 1. Changes in pH on deterioration

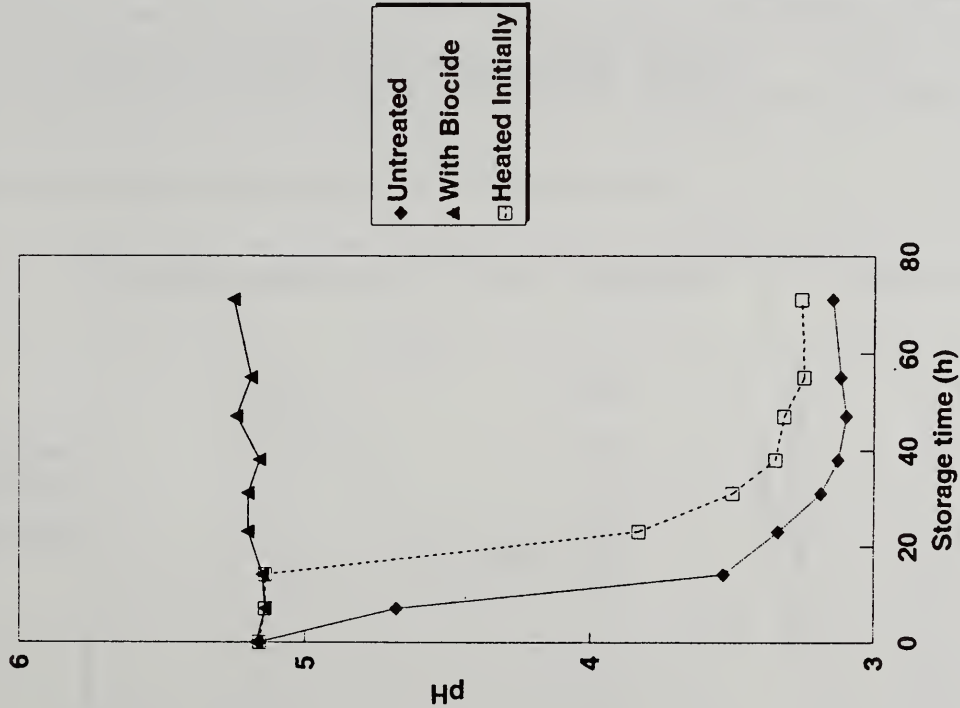
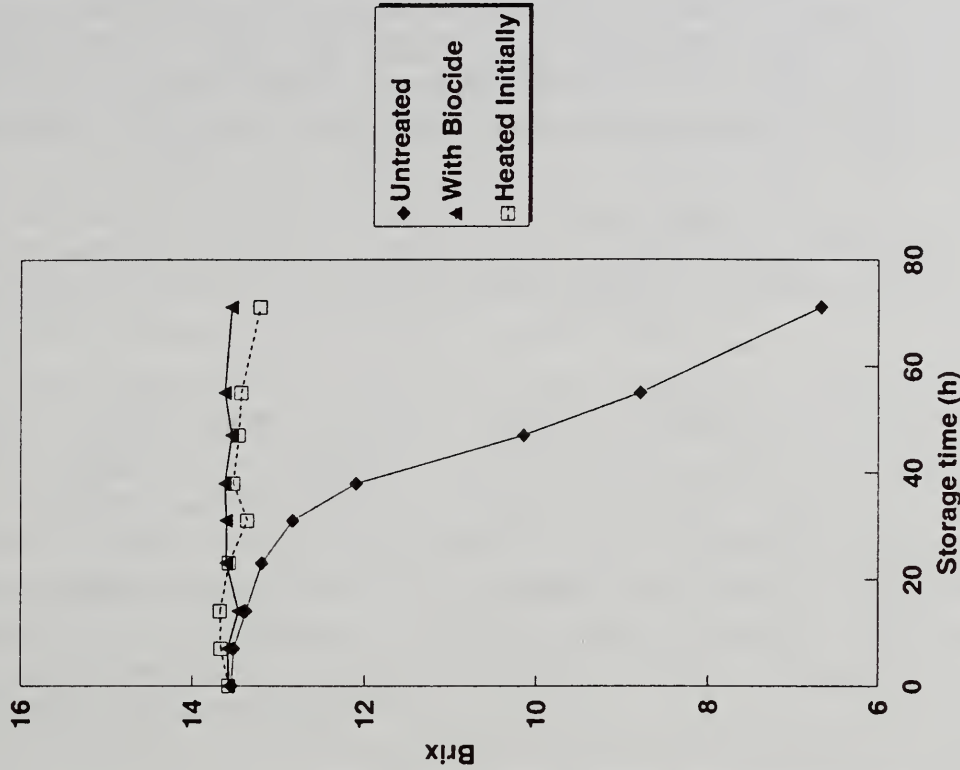
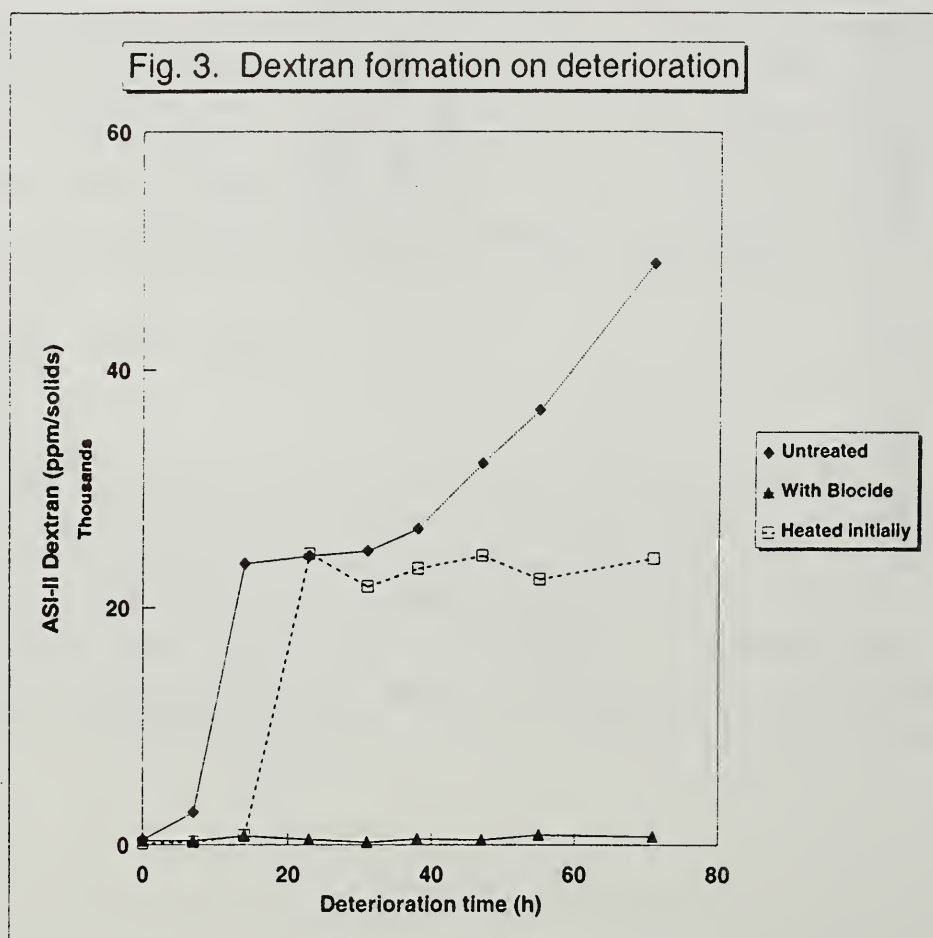


Fig. 2. Changes in Brix on deterioration



$^{\circ}\text{Brix}$ also changed dramatically in the juices on deterioration, as illustrated in Figure 2. $^{\circ}\text{Brix}$ of the untreated juice decreased slightly over the first 23 h, but the rate of $^{\circ}\text{Brix}$ reduction then accelerated, indicating solids were being utilized as a biomass source and converted into a liquid and/or gas state. In comparison, the $^{\circ}\text{Brix}$ of the heated juice decreased only slightly on deterioration suggesting that deterioration, especially after 14 h, was because of the metabolic change of biomass into another solid source. In the biocide control juice, there was no significant change in $^{\circ}\text{Brix}$ across 71 h.

High and low molecular weight dextran polysaccharide formation in the three juice treatments is shown in Figure 3. In the untreated juice, dextran formation was slow in the first 7 h, then accelerated between 7-14 h. A slowdown then occurred up to 31 h, and then a second acceleration phase (this may just be characteristic of the microbial load). In contrast, in the pre-heated juice, no dextran was formed in the first 14 h, because the heat would have destroyed or vastly reduced the numbers of most of the *Leuconostoc* bacteria initially present in the juice. The large formation of dextran between 14-23 h could be because of re-inoculation in the incubator from the non-sterile experimental conditions. A further explanation is that the heat treatment just reduced the number of viable of *Leuconostoc* bacteria to a level where lag phase growth occurred, and it took 14 h for the bacteria to recuperate and produce dextran, especially in exponential phase growth. After 23 h no significant dextran was formed which was likely because the very low pH stopped *Leuconostoc* growth and/or the activity of dextransucrase. As expected, in the biocide control juice, there was no formation of dextran across 71 h. Ravelo, et al., (1995) applied the disinfectant IFOPOLTM to stored billeted cane and observed that the formation of polysaccharides, as well as total oligosaccharides was greatly reduced.



Changes in sucrose, glucose, and fructose concentrations on a °Brix basis are illustrated in Figure 4. Degradation of sucrose in the factory can occur via a variety of mechanisms. It can be hydrolysed into glucose and fructose by either acid (acid inversion of sucrose) or by naturally occurring cane invertase enzymes (sucrose inversion). Another mechanism of sucrose loss is by its utilization by microbes. High infections and stagnant zones occur often in the cane factory, particularly in the milling station, and these act as 'open fermentors'. *Leuconostoc* bacteria are able to utilize the glucose in the sucrose molecule to form dextran (a glucose polysaccharide). Yeast, particularly *Saccharomyces* often found at factories (Chen and Chou, 1993), can convert sucrose into ethanol and carbon dioxide, especially under anaerobic conditions often found in cane storage piles and at the factory. Yeast, and other microbes, are also known to secrete periplasmic invertase enzymes (Hanko and Rohrer, 2000).

In the untreated juice, sucrose degraded rapidly (Figure 4a), particularly over the first 14h (29.0% sucrose loss), which is further evidenced by the concomitant, sharp increase in glucose and fructose concentrations (Figures 4b and c). Although, after 39 h, sucrose loss decelerated, by 71 h very little sucrose, glucose, and fructose remained, because the solids had been transformed by microbes (see °Brix results). In comparison, the sucrose in the biocide treated juice was only slightly degraded in the juice (1.7% after 14 h). This slight degree may be because the biocide is unable to stop the enzymic and acid inversion of sucrose. In the juice pre-heated before deterioration, only 0.4% sucrose was measurably lost during the first 14 h.

This strongly suggests that the heating treatment denatured the invertase enzymes as well as markedly reduced the levels of microbes (including thermophilic bacteria), and that at ambient temperatures, acid sucrose inversion contributes very little to sucrose loss in the factory. Glucose and fructose similarly increased slightly on sucrose inversion and G/F ratios stayed constant (Table 1).

Table 1. Effect of deterioration time on glucose/fructose ratios

Deterioration time (h)	Unadulterated Juice	Juice with Biocide	Heated Juice
0	1.08	0.99	1.10
7	0.88	1.022	1.11
14	0.65	1.02	1.12
23	0.74	1.04	0.42
31	0.79	1.04	0.12
38	0.79	1.03	0.15
47	0.49	1.03	0.14
55	0.39	1.03	0.09
71	0.05	1.03	0.12

Fig. 4a. Sucrose losses on deterioration

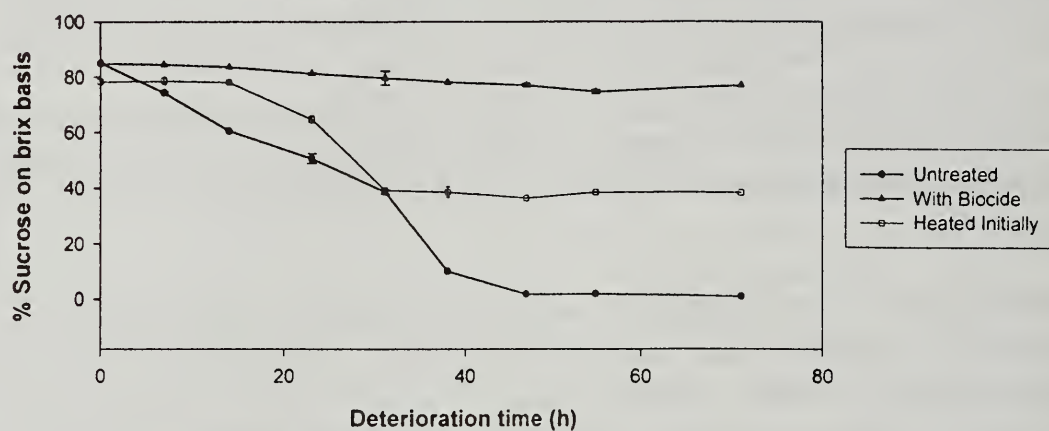


Fig. 4b. Glucose changes on deterioration

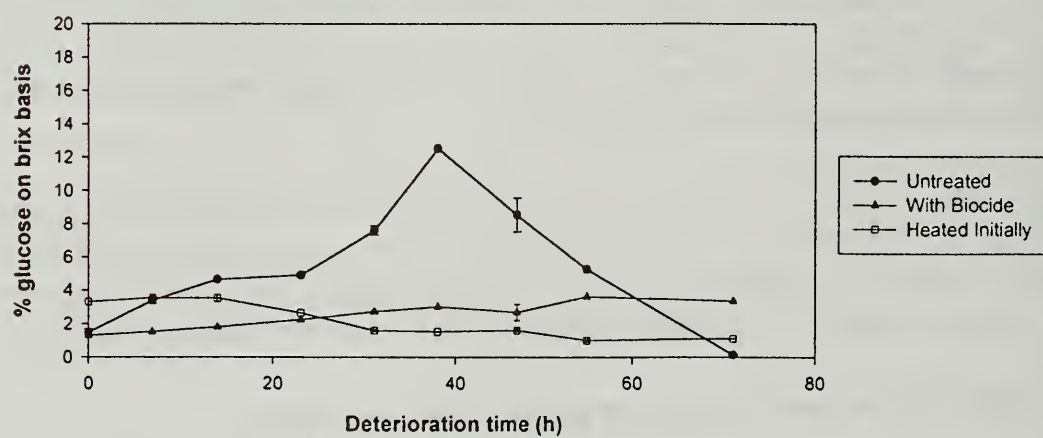
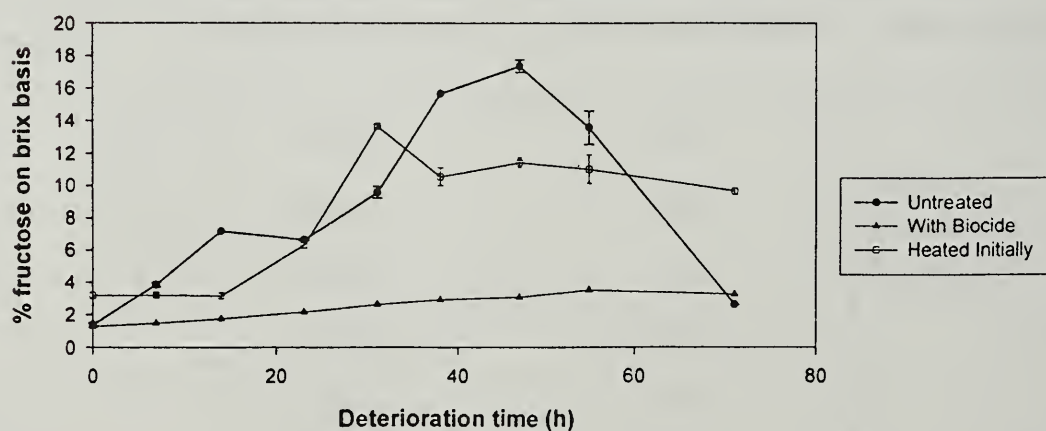


Fig. 4c. Changes in fructose on deterioration



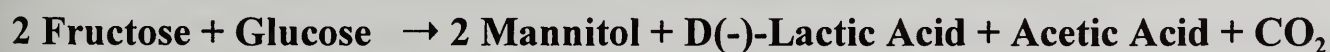
Using the combination of untreated, biocide-treated, and heat-treated juices after 14 h deterioration, it was possible to calculate the contributions of the different sucrose loss mechanisms. (Note: retention time in the factory is in minutes not hours). The untreated juice was taken as equivalent to total deterioration, biocide treated juice as equivalent to enzymic and chemical deterioration, and the pre-heated juice as chemical (acid) deterioration only. It was calculated that 93.0% of deterioration was microbial, 5.7% enzymic, and 1.3% was chemical. As microbiological deterioration is such a major source of loss, the need to use biocide agents, or other aseptic conditions at the factory is highlighted.

Dextran formation on deterioration of the untreated juice was also indicated by the change in G/F ratios (Table 1). Low G/F ratios indicate a relative increase in fructose to glucose, which occurs when dextran is formed because *Leuconostoc* bacteria utilizes glucose to form dextran, leaving fructose from the sucrose molecule as a by-product.

Sensitive Indicators of Cane Deterioration

The author previously developed and used an ion chromatography with pulsed amperometric detection (IC-IPAD) method (Eggleston et al, 2001b) to study deterioration products of cane, with an emphasis on oligosaccharides. Oligosaccharides are known to form in deteriorated cane during delays between cutting and crushing, but their formation has been usually attributed to microbial (mainly bacterial) activity (Ravelo et al, 1991, 1995) and enzymic activity (Morel du Boil, 1995). However, kestose oligosaccharides, which can form from enzymic activity in the cane, are also degradation products from the acid degradation (inversion) of sucrose (Richards, 1988) and are, therefore, formed from chemical reaction activity as well. The major oligosaccharides formed on cane deterioration (Morel du Boil, 1995; Eggleston et al, 2001b) are from the kestose family: 1-kestose (GF₂), 6-kestose (GF₂), neo-kestose (GF₂), nystose (GF₃), and kestopentaoses (GF₄) and kestohexaoses (GF₅) isomers, as well as oligosaccharides formed as acceptor (secondary) products (Robyt & Eklund, 1982; Demuth, *et al.*, 1999) from the action of dextranase in *Leuconostoc* strains. Examples of dextran associated oligosaccharides are isomaltotriose, isomaltotetraose, leucrose, and palatinose.

Mannitol is also formed from fructose by the lactic acid *Leuconostoc* bacteria (Soetart, 1991). Fructose, as an alternative electron acceptor, is reduced to mannitol by the enzyme mannitol dehydrogenase. During this process, the reducing equivalents are generated by the coupled conversion of glucose into D(-)-lactic acid and acetic acid. The following theoretical fermentation equation was derived by Vandamme, *et al.*, 1996:



Mannitol has recently been identified (Steinmetz et al, 1998) as a sensitive quality criterion for frost damaged sugarbeets and, consequently, was analyzed in this study to ascertain if it could be used to indicate cane deterioration.

Ethanol has also been advocated as a cane deterioration indicator (Lionnet & Pillay, 1988; Lionnet, 1996), particularly in burnt whole-stalk cane (Lionnet & Pillay, 1987). It is a metabolic by-product of many microbial reactions, and the amount formed depends on the type of microbe, as well as microbial growth parameters including temperature and humidity. Ethanol is a major by-product of yeast fermentation reactions, with yeast converting sucrose into ethanol and carbon dioxide, especially under dry and anaerobic conditions. It was stated by Mackrory et al, (1984) that *Leuconostoc* bacteria, besides forming dextran, can also be heterofermentative and produce lactic acid, ethanol and carbon dioxide, although Lillehoj et al, (1984) and Erten (1998) reported that this is only the case if glucose, not sucrose, is the carbohydrate carbon source.

Recently, Hanko & Rohrer (2000) used an IC-IPAD method to simultaneously analyze for sugars, sugar alcohols (alditols), and alcohols produced by growing yeast (*Saccharomyces cerevisiae*) cultures and, in this study the author was able to use an IC-IPAD method to simultaneously detect oligosaccharides, mannitol (alditol), and ethanol cane deterioration products. The IPAD method is relatively insensitive to ethanol, compared to mannitol, oligosaccharides, and other sugars. However, this may be an advantage when large concentrations of ethanol are forming on microbial deterioration.

There were dramatic changes in the IC-IPAD chromatograms of the untreated juice across 71 h, which are illustrated in Figure 5. Even after the first 7 h, mannitol and isomaltotriose had increased, and leucrose was visible, confirming that mannitol dehydrogenase and dextransucrase activities were present. However, ethanol had also formed slightly and the °Brix had begun to decrease (see Figure 2) which strongly suggests that *Leuconostoc* bacterial deterioration and other microbial (most likely yeast reactions are involved because of the reduction in °Brix) deterioration reactions were simultaneously occurring in the juice. As expected, for the biocide treated juice, *Leuconostoc* metabolites including mannitol and isomaltotriose, within experimental error, did not form over the 71 h deterioration time, and leucrose could not be measured (see Figure 6); furthermore, no additional ethanol was formed, and even the initial concentration decreased, which may be because of evaporation. However, the trisaccharide kestoses, neo-, 6-, and 1-kestose, increased across the 71 h which further confirms that the enzyme and acid inversion of sucrose makes a small, but significant, contribution to cane deterioration.

In the pre-heated juice, none of the deterioration products studied increased over the first 14 h (Figure 7), confirming previous results that the initial heat treatment delayed deterioration. After 14 h, however, there was a marked increase in dextransucrase metabolites including isomaltotriose, isomaltotetraose, leucrose, and palatinose. Moreover, as can be seen in Figure 7, mannitol also produced by *Leuconostoc* bacteria, but via a different mechanism to dextran and dextransucrase acceptor products, increased even more dramatically. As the rate of formation of mannitol was much higher than the other metabolites, including ethanol (see Figure 7), it would make a sensitive indicator for cane deterioration that could be used by factory staff to check if the load of cane delivered to the factory is going to cause dextran associated problems in subsequent processing. Moreover, there were very high correlations between mannitol and dextran in the pre-heated ($R^2=0.95$, $P<.005$) and untreated ($R^2=0.98$, $P<.015$) juices, with the latter being illustrated in Figure 8. Results here warrant an investigation into the use of mannitol as a cane deterioration indicator under industrial conditions.

Fig. 5. IC-IPAD chromatograms of the untreated juice on deterioration

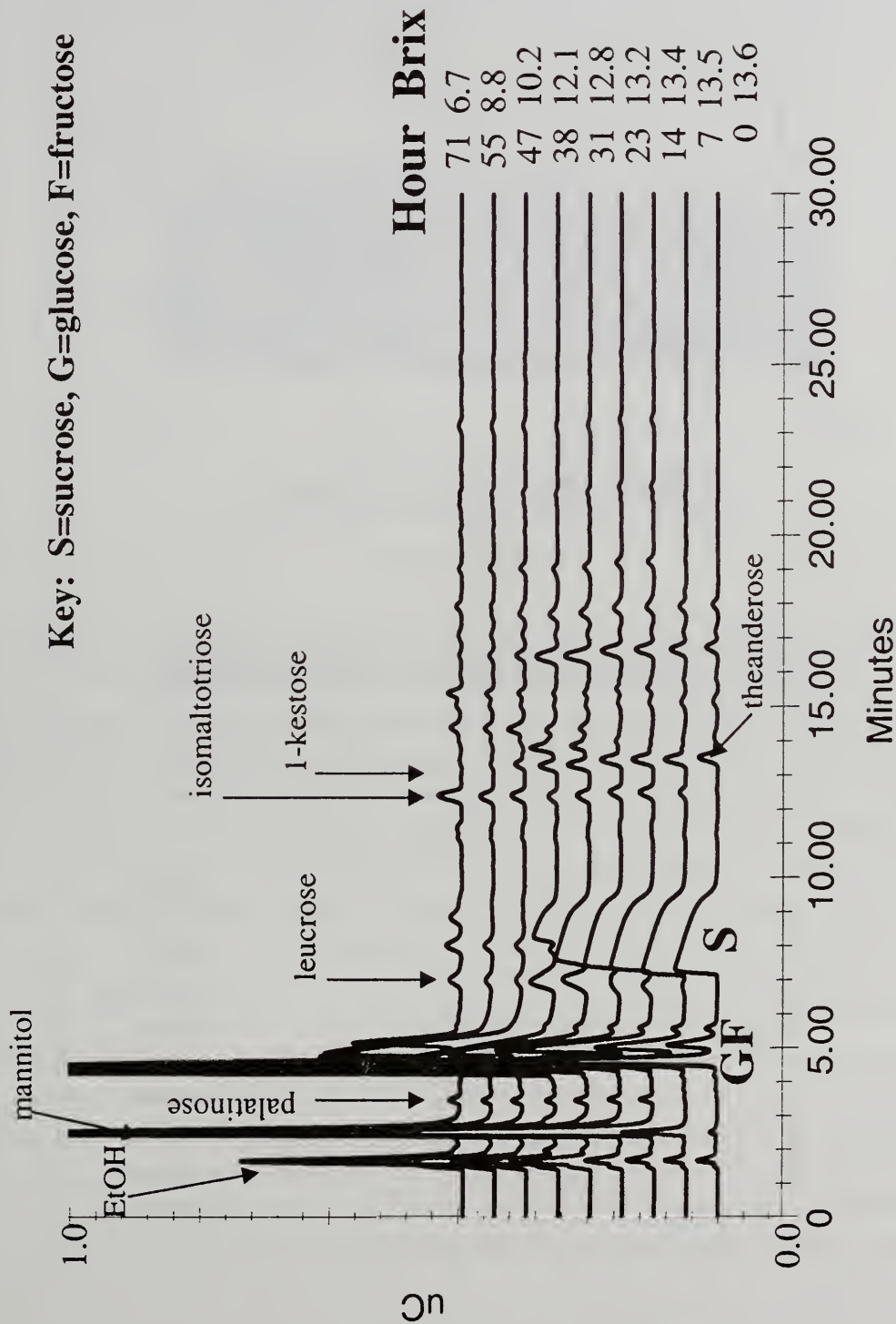


Fig. 6. Deterioration products in biocide treated juice

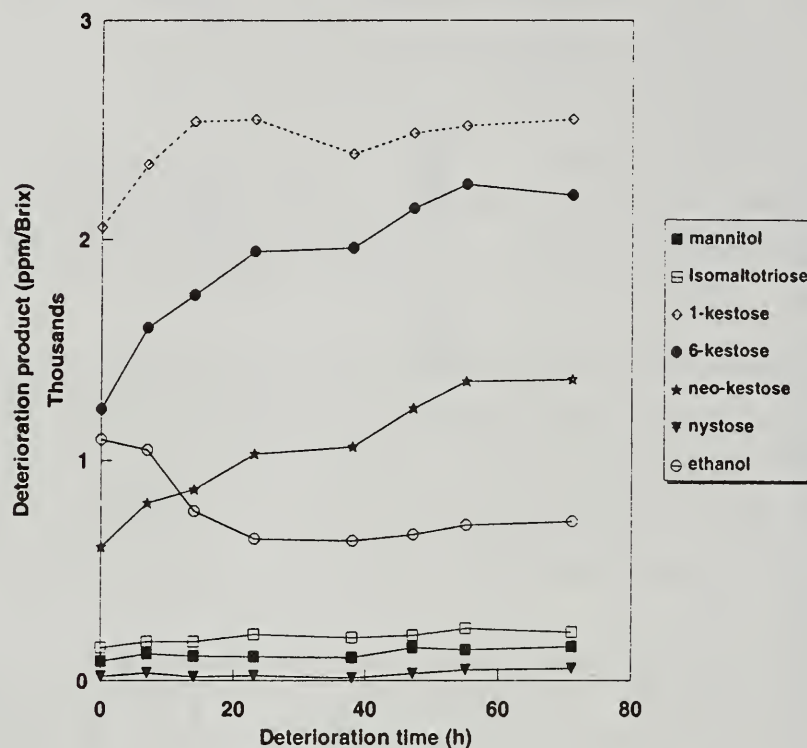


Fig. 7. Deterioration products in heat treated juice

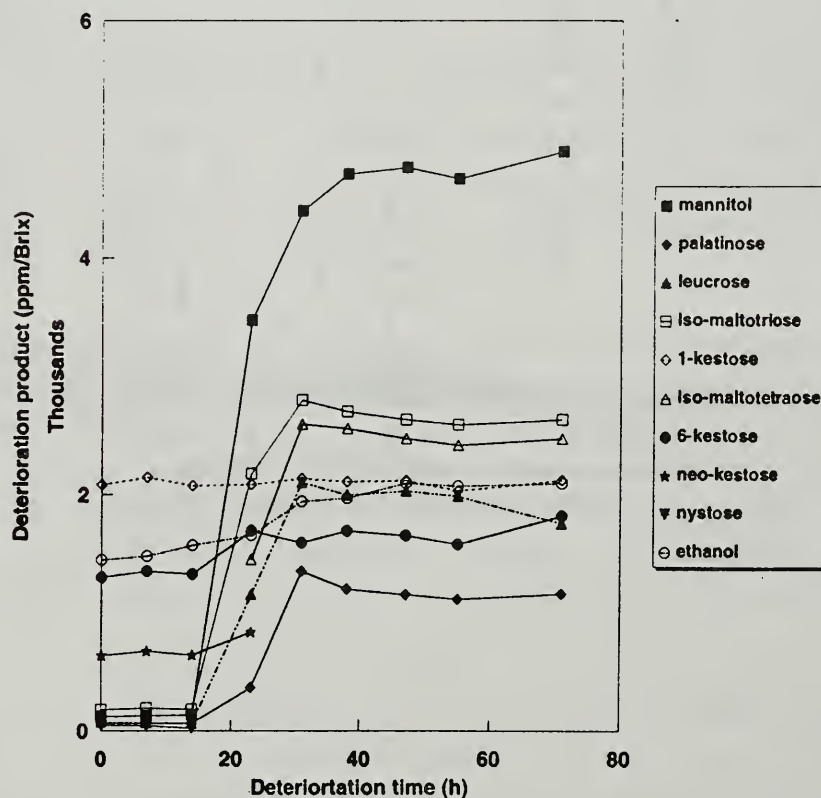
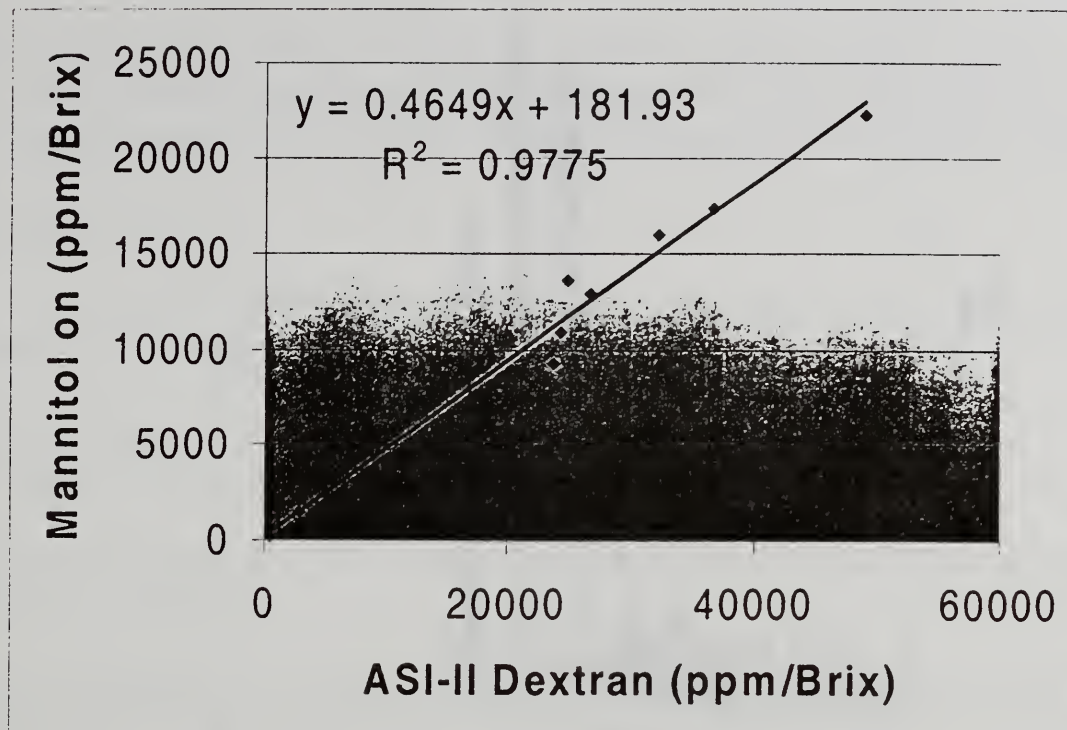
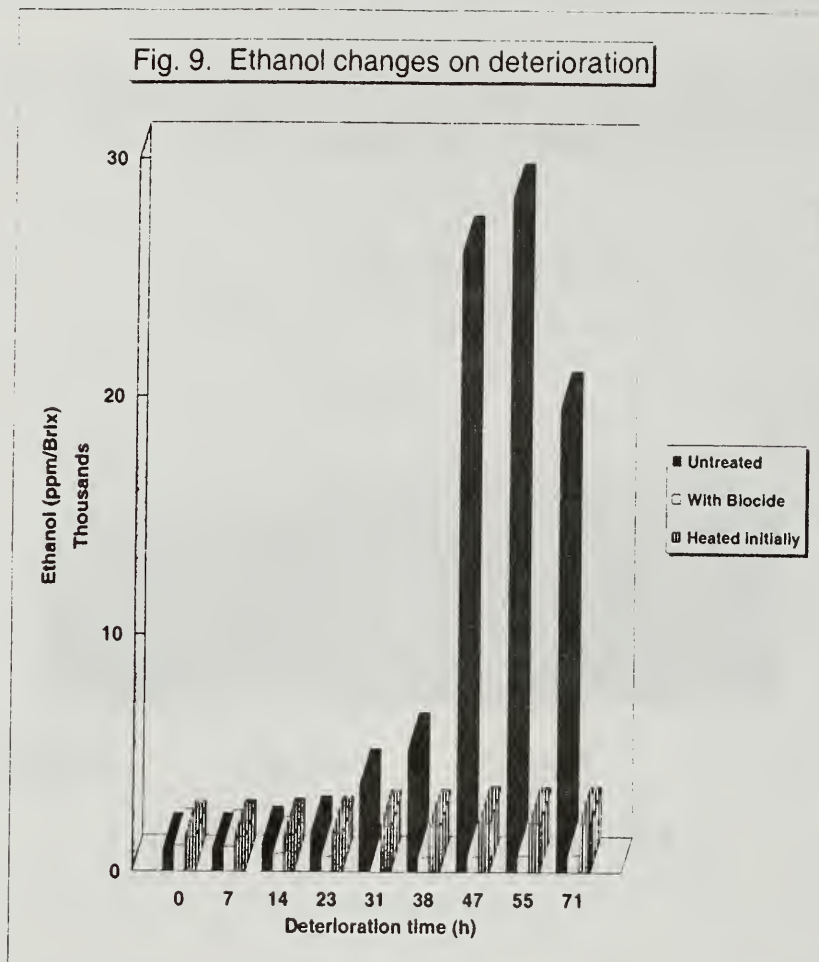


Fig. 8. Strong correlation between dextran and mannitol in untreated cane juice



In marked contrast to the formation of dextran in the pre-heated juice after a 14 h delay, there were no increases in the concentrations of sucrose inversion products, the trisaccharide kestoses. This confirms that inversion of sucrose at ambient temperatures is more attributable to enzymic rather than acid inversion (the pH was very acidic at this stage of deterioration). After 14 h deterioration of the pre-heated juice, results have indicated that the sample was either re-inoculated in the non-sterile incubator or *Leuconostoc* bacteria growth recuperated. A surprising observation was that, even though after re-inoculation dextran, dextran associated oligosaccharides, and mannitol formed, only a relatively small amount of ethanol formed (Figures 7 and 9). This strongly suggests that ethanol is only slightly associated with dextran formation in cane juice by *Leuconostoc* bacteria, which may be because it is formed only when glucose is the carbohydrate carbon source (Lillehoj, *et al.*, 1984). Furthermore, in strong contrast to the heat and biocide treated juices, marked ethanol formation occurred in the untreated juice (see Figures 5 and 9). The untreated juice clearly had associated yeast fermentation, dextran forming, and other microbial deterioration reactions. Most processing problems, particularly in the U.S., including increased viscosity reducing flow throughput and slowing of filtration and crystallization rates, are because of the dextran polymer. Ethanol, as suggested from results in this study, is more an indicator of yeast and possibly other, non *Leuconostoc*, bacterial deterioration in cane and, therefore, cannot always predict processing problems associated with dextran.



MAJOR CONCLUSIONS

- ▶ By comparing the deterioration (at 27°C) of heat and biocide treated cane juice with untreated juice, it was possible to quantitatively compare the relative contributions of microbial, enzymic, and chemical deterioration on sucrose losses. Over the first 14 h of deterioration, 93.0% of sucrose deterioration was microbial, 5.7% enzymic and 1.3% was chemical (acid degradation).
- ▶ IC-IPAD can be used to simultaneously analyze for ethanol, mannitol, and oligosaccharides in deteriorated cane juice.
- ▶ The rate of formation of mannitol, produced from the reduction of fructose by mannitol dehydrogenase in dextran forming *Leuconostoc* bacteria, was much higher than associated oligosaccharides or ethanol formation. A further investigation of the use of mannitol as a sensitive indicator of future dextran related processing problems at the factory is warranted.
- ▶ Mannitol formation was strongly correlated with the formation of high and low MW dextran. Studies are underway to assess mannitol's worth as a measure of dextran in core and crusher juices at the factory.
- ▶ Ethanol was shown not to be very useful as an indicator of cane dextran deterioration.
- ▶ There is now a need to undertake further deterioration studies with different sources of cane juice and specific inoculations, with yeast and *Leuconostoc* bacteria which are frequently found in cane juice.

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MANNITOL AND OLIGOSACCHARIDES: POTENTIAL NEW CRITERIA FOR DETERMINING COLD TOLERANCE IN SUGARCANE VARIETIES

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ABSTRACT

Sugarcane can be very susceptible to damage by freezes. The frequent winter freezes of Louisiana force the industry to adapt to a short growing season (7 to 9 months) and a short milling season (approx. 3 months). Freeze deteriorated cane can cause problems in processing and sometimes leads to a factory shut-down. This study was undertaken during the 2000/2001 harvest season to assess the cold tolerance performance of six commercial sugarcane varieties and to establish new and more sensitive criteria to measure cold tolerance. Two commercial varieties CP 70-321 and CP 79-318 with known cold tolerance were planted in the study as controls. The other varieties included LHo 83-153, LCP 85-384, HoCP 85-845 and HoCP 91-555. Freezing temperatures occurred on Dec 20, 2000 when the minimum field temperature was 24° F, and again on Dec 21, Dec 30 through Jan 5, 2001, Jan 9-10 and January 20-21. The lowest field temperature recorded was 22° F on Jan 4. Freezing conditions prevailed for 8-15 hours during each freeze incident. Stalks of all varieties were frozen to the ground following the initial freeze with freeze cracks evident only after the January 4 freeze. For this study, samples were taken on the date of the first freeze, December 20, and subsequently again at 7, 14, 22 and 30 days after the first freeze. Criteria used to measure overall stalk cold tolerance included changes in pH, Brix, dextran (ASI-II method), sucrose, glucose, and fructose concentrations. Mannitol, ethanol and the oligosaccharides palatinose, leucrose, isomaltotriose and 1-kestose were simultaneously measured using IC-IPAD. Marked differences were observed for most criteria for all varieties, particularly 22 and 30 days after the first freeze. Mannitol was strongly correlated ($r^2 = 0.84$) with dextran confirming its use as an indicator for cane dextran

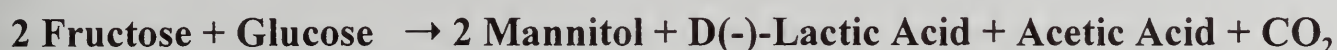
or *Leuconostoc* deterioration. In comparison, ethanol was only weakly correlated ($r^2=.55$) with dextran and did not always predict cane dextran deterioration. Iso-maltotriose was the most sensitive oligosaccharide indicator of freeze deterioration, although both leucrose and palatinose could be used to confirm if severe dextran formation ($>1500\text{ppm/Brix}$) has occurred in cane. Iso-maltotriose was strongly correlated ($r^2 = 0.89$) with dextran and pH ($r^2 = 0.91$). pH was also a strong indicator of both dextran ($r^2 = -0.85$) and mannitol ($r^2 = -0.92$) formation. Four of the varieties, CP 79-318, LCP 85-384, HoCP 85-845 and HoCP 91-555, were shown to be susceptible to other sources of microbial and enzymic deterioration as well as dextran deterioration from *Leuconostoc* bacteria, especially 30 days after the first freeze. This was indicated by increased glucose/fructose ratios, ethanol formation, changes in 1-kestose concentration, and further sucrose losses.

INTRODUCTION

It is well known that sugarcane can be very susceptible to damage by freezes and freeze deteriorated cane can cause problems in processing and sometimes leads to a factory shut-down. The exposure of sugarcane to damaging frosts occurs in over 20 of the 70 sugarcane producing countries, but is most frequent on the mainland of the United States. The frequent winter freezes of Louisiana force the industry to adapt to a short growing season (7 to 9 months) and a short milling season (approx. 3 months). The nature and extent of damage to cane depends on the type and number of periods of the frost, which may be light, mild, or severe (Naqvi and Alam, 1976). Damage from a freeze can be even more severe when warm, wet weather, which is ideal for microbial growth, follows the freeze. Generally, cane damaged by a severe freeze produces juices of lower purity, higher acidity and abnormal amounts of polysaccharides, especially dextran (see Legendre et al, 1985). Following freeze injury, dead and moribund cells become vulnerable to the invasion of microbes, particularly *Leuconostoc* bacteria which are mostly responsible for the formation of dextran by dextranase enzyme. The entry of microbes into cane tissue is facilitated by dead lateral buds (24°F) and by freeze cracks (22°F). Irvine and Legendre (1985) proposed two mechanisms for deterioration: 1) susceptibility of tissue to freezing, and 2) susceptibility to microbe invasion and subsequent polysaccharide formation after the freeze.

Because of the prevalence of damaging frosts in Louisiana, historically great emphasis has been placed on breeding for "cold tolerant" or frost resistant cane varieties. Legendre, *et al.*, (1985) showed that there was a varietal effect on level of dextrans and total polysaccharides in cane left in the field after freeze damage. Such breeding programs are highly reliant on quality criteria/indicators to allow proper selection and development of cold tolerant varieties. Sugarcane agronomists and technologists have reported a variety of physico-chemical criteria to measure cane deterioration after a freeze. Changes in juice pol, titratable acidity and dextran content were reported by Legendre, *et al.*, (1985) to be the most useful criteria. However, not all criteria (which are mostly formed deterioration products) impact future factory processing, and some are not as direct or sensitive as required, while others are difficult or too time consuming to measure. New and more sensitive criteria for levels of freeze deterioration need to be established in order to aid the breeding programs, and better predict the quality of the cane to be processed as well as the effect on process conditions.

In the last decade, oligosaccharides have been reported as indicators of deterioration in stored cane, particularly burnt billeted cane (Ravelo, *et al.*, 1991, Morel du Boil, 1995 and Eggleston, *et al.*, 2001a,b). Like the dextran polysaccharide which has negative impact in processing mostly associated with increased viscosity effects, certain oligosaccharides directly and negatively impact the efficiency of factory processing as they can interfere with crystallization (Morel du Boil, 1995), deforming the crystal shape (crystal elongation). Recently, Eggleston (2002) also showed that mannitol could be a very sensitive indicator of dextran deterioration in stored, deteriorated cane juice and Steinmetz, *et al.*, (1998) previously observed that mannitol was strongly correlated with the quality of frost-damaged sugarbeets. Furthermore, mannitol has been known to increase the viscosity of sugar syrups making them difficult to recover sucrose from (Bliss, 1975). Like dextran, mannitol is also formed by the lactic acid *Leuconostoc* bacteria (Soetart, 1991), but unlike dextran which is formed by the dextransucrase enzyme, mannitol is formed by the enzyme mannitol dehydrogenase, which is illustrated in the following theoretical fermentation equation (derived by Vandamme, *et al.*, 1996):



Fructose can also be simply reduced to mannitol by mannitol dehydrogenase NADH linked activity in *Leuconostoc* bacteria (Grobben, *et al.*, 2001).

Consequently, in this study various known and new quality parameters including oligosaccharides and mannitol were studied to find more reliable predictors of freeze deteriorated cane in six commercial sugarcane varieties. This information could then be used by breeders to aid cold tolerance breeding programs, and by processors to assess the quality of cane being processed.

EXPERIMENTAL

General Sampling

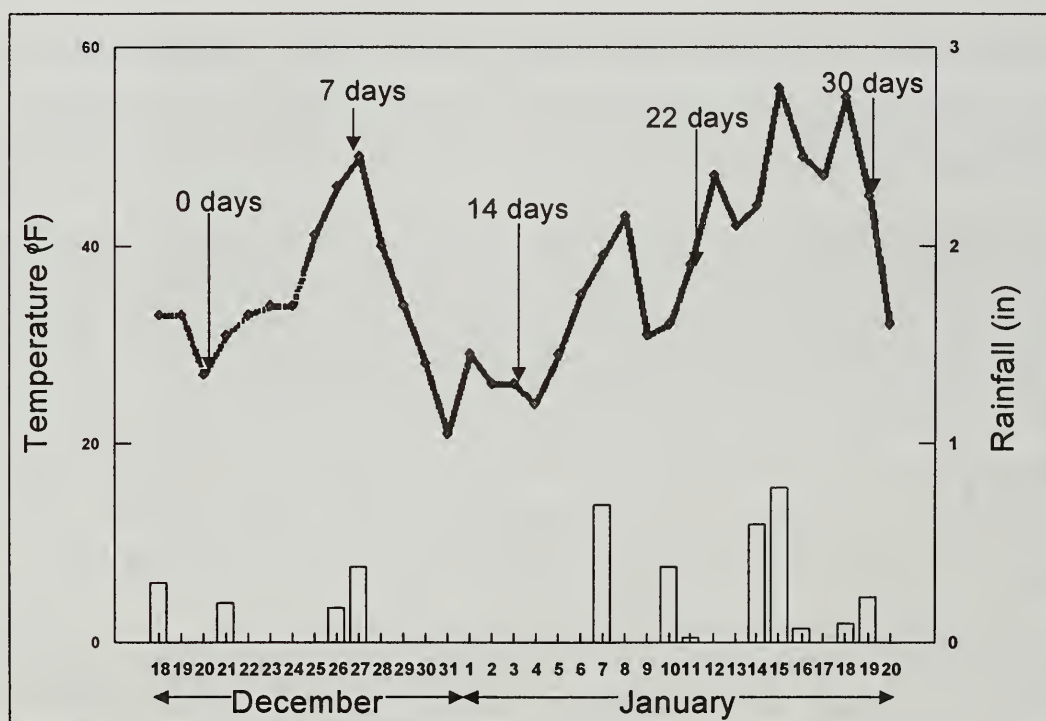
Field experiments consisting of 3-row plots are routinely planted at the Ardoyne Farm of the USDA-ARS-SRRC at Houma, Louisiana for the estimation of stalk cold tolerance of commercial and candidate varieties. In this study, commercial varieties of known cold tolerance were grown as controls and included CP 70-321 for good cold tolerance (Irvine and Legendre, 1985) and CP 79-318 which is known for poor cold tolerance. Six commercial varieties were planted which included the two control varieties; the other varieties were LHo 83-153, LCP 85-384, HoCP 85-845 and HoCP 91-555. Planting occurred on August 27, 1999, and was done on raised ridges 6 ft apart. Variety plots were approx. 50 ft long and 3 rows wide. The experimental design was a randomized complete block with 4 replications. Plots were cultivated and fertilized according to recommended plantation practices; insecticides were applied as required according to the economic threshold (Legendre, 2001). The cane was allowed to remain in the field until December 18, 2000 which was just before the first freeze of the harvest season in the year following planting. Just prior to or immediately following the freeze, five samples were removed serially along the center row of each plot. Each sample consisted of 10 stalks cut at the ground by hand but not stripped or topped of leaves. The 10-stalk sample was passed once through a 3-roller sample mill. A sub-sample mill juice was then immediately taken for dextran (ASI-II method) analysis. The rest of the juice was frozen and then transported to the analytical laboratory. Composite juices of each variety for each sampling date were

made by physically combining 15 g of each of the 4 replicates. The biocide sodium azide (0.02%) was added immediately to each composite to prevent any further microbial deterioration from occurring. Juices were stored in a -40 °C laboratory freezer until analyzed.

Freezes During the 2000/2001 Cane Harvest and Sampling Dates

Freezing temperatures that affected the Louisiana sugar industry during the 2000/2001 harvest occurred on December 20-21 and 30-31, 2000 and January 1-5, 9-10, 20-21 and 23, 2001. The official temperatures recorded at the Houma research station near to where the Ardoyne farm is located are illustrated in Figure. 1.

Fig. 1. 2000/2001 weather data and sampling dates^{a,b}



^a For USDA-ARS Research Station, Houma, LA, USA

^b Cane was harvested a day earlier than juice extraction

On December 20, subfreezing temperatures were reported for more than 12 h with a min. field temperature of 24° F reported at Houma (Figure 1). No freeze cracks were apparent although most stalk tissue showed signs of being frozen and the top portion of most stalks (18-24 in) had begun to soften and wilt. The coldest field temperatures (field temperatures were usually lower than the official temperatures recorded at nearby Houma research station shown in Fig. 1) occurred during the period January 4-11 and the temperature dipped to 22° F at Houma on January 4 (Figure 1). On January 10 it was observed that freeze cracks had occurred in all varieties with juice leakage from the cracks as well as from the axillary buds. It was evident from field investigations that serious tissue damage occurred within the stalks following the January 4 freeze. Sampling dates for this study are indicated in Fig. 1. Samples were collected on December 18 and processed on December 20 (control samples prior to the first freeze) and December 27, 2000, January 3, 11 and 19, 2001.

Ion Chromatography with Pulsed Amperometric Detection (IC-IPAD) Analysis of Carbohydrates and Sugar Alcohols. See Eggleston (2002) for method. All samples were °Brix adjusted to the lowest sample °Brix before diluting (1g/25ml) in order to compare chromatograms directly. Oligosaccharides, mannitol, and ethanol were quantitated in reference to raffinose, mannitol, and ethanol standards, respectively.

Sucrose, Glucose and Fructose by Gas Chromatography (GC). See Eggleston (2002) for method.

Dextran. Duplicate samples on each replicate were analyzed for dextran using the ASI (Audubon Sugar Institute) II method (Sarker & Day, 1986) which uses dextranase enzymes. Average results are reported.

°Brix. The mean °Brix of triplicate samples was measured using an Index Instruments TCR 15-30 temperature controlled refractometer accurate to ± 0.01 °Brix.

pH. Measurements of pH were at room temperature ($\sim 25^{\circ}\text{C}$), using an Ingold™ combination pH electrode calibrated at room temperature using two different pH buffers (pH 7 and 10). The electrode was connected to a Metrohm 716 DMS pH meter.

Statistical Correlations. Pearson correlation coefficients were calculated to investigate relationships among the various deterioration criteria (N=30) using PC-SAS 6.12 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Changes in pH

Changes in pH amongst the varieties are listed in Table 1. Organic acids, particularly lactic acid, are produced during cane deterioration from the degradation of sugars and cause a reduction in pH. However, pH is not usually considered a sensitive measure for deterioration because the buffering capacity of the juice reduces this pH change on deterioration. Nevertheless, there was a marked difference in pH decrease with post-freeze date, particularly 22 and 30 days after the initial freeze.

Dextran Formation

The ASI-II method used to determine dextran in this study measures both high and low molecular weight dextran and results are illustrated in Figure. 2. Marked differences in dextran formation were observed amongst the varieties, particularly 22 and 30 days after the initial freeze when freeze cracks were visible. Variety LCP 85-384 was susceptible to dextran formation even after the first freeze (at 0 days), and HoCP 91-555 was susceptible 7 days later. Varieties CP 70-321 and LHo 83-153 were the least susceptible to *Leuconostoc* invasion and subsequent dextran formation, even after 22 days. Results suggest that the final freeze which caused cracks to appear on the cane stalks, accelerated the invasion of the wounded cane by *Leuconostoc* bacteria and the utilization of sucrose for dextran formation.

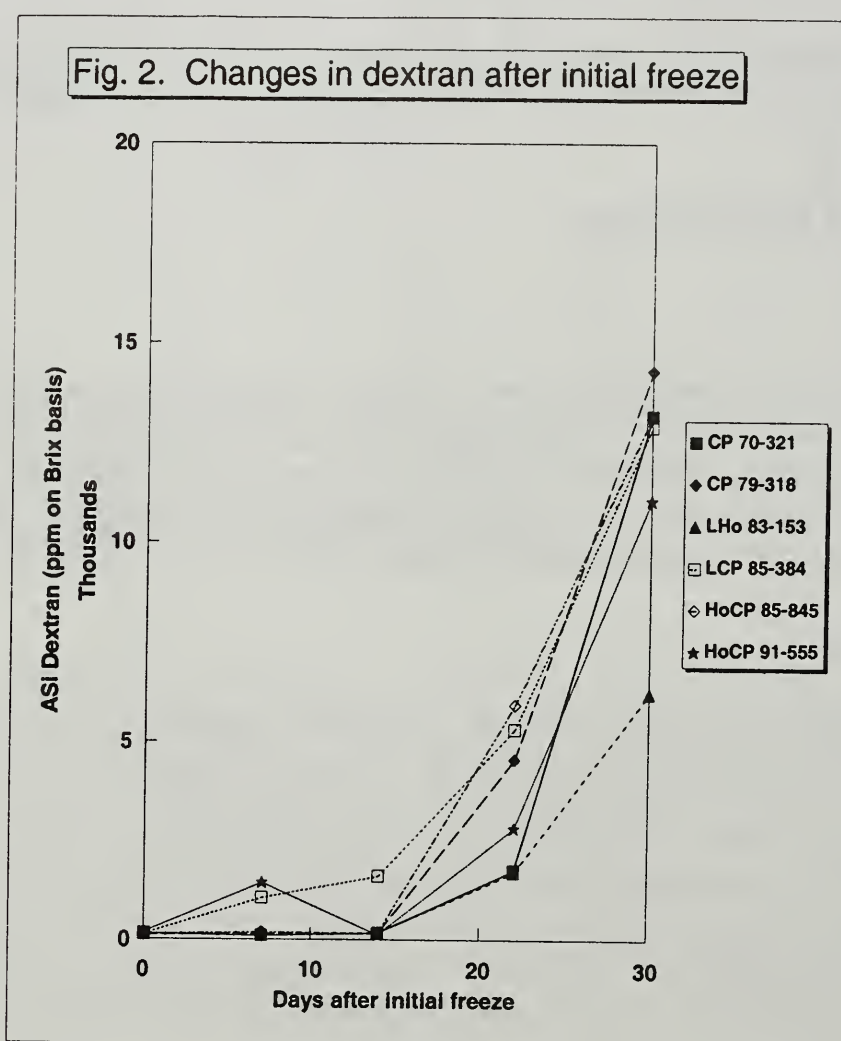
Table 1. Effect of days after initial freeze on the pH of the cane juices.

Cane Variety	pH				
	0 days	7 days	14 days	22 days	30 days
CP 70-321	5.38	5.36	5.41	5.34	4.32 ^b
CP 79-318	5.26	5.35	5.35	4.60 ^a	3.99 ^b
LHo 83-153	5.36	5.28	5.32	4.90 ^a	4.08 ^b
LCP 85-384	5.29	5.30	5.30	5.12	4.32 ^c
HoCP 85-845	5.30	5.32	5.34	4.72 ^a	4.18 ^b
HoCP 91-555	5.40	5.37	5.47	5.24	4.18 ^b

^a the natural brown juice color had turned yellow

^b yellow and smells of alcohol

^c yellow with slight smell of alcohol



Effect of Freezes on Sucrose, Glucose and Fructose Concentrations

Freezes are known to kill and rupture cells which causes sucrose inversion to occur, because of higher acidity levels and increased activity of invertase enzymes. The latter could be due to increased mobilization of cell invertases, the possible synthesis of a wound induced invertase, and/or because of decreased activity in sucrose synthesis enzymes induced by the pH changes (personal communication, S. Lingle). Furthermore, the wound sites and associated cell rupture allow microbes to invade and utilize sugars such as sucrose. The effect of freezes on sucrose, glucose and fructose concentrations are listed in Table 2.

Initially (0 days) there were slight differences in sugar concentrations amongst the six varieties. CP 79-318 and HoCP 91-555 had the lowest sucrose and highest glucose and fructose. Minor increases in glucose and fructose 7 days after the initial freeze indicated that the most varieties had been susceptible to slight deterioration after the first freeze, with HoCP 91-555 being particularly susceptible as indicated by the concomitant increase in dextran after 7 days (Figure 2). Subsequently, marked degradation of sucrose with formation of glucose and fructose occurred particularly 22 and 30 days after the initial freeze (Table 2). This is further confirmation that the freeze of Jan 4 (equivalent to day 15 after the initial freeze) when freeze cracks were visible, caused dramatic deterioration to occur in all cane varieties, although varietal differences were still apparent.

Table 2a. Sucrose "True Purity" Concentrations

Cane Variety	% SUCROSE (on Brix Basis)				
	0 days	7 days	14 days	22 days	30 days
CP 70-321	92.40	92.89	92.44	87.69	75.60
CP 79-318	88.42	88.91	88.76	78.12	54.34
LHo 83-153	91.26	92.0	88.56	88.04	77.32
LCP 85-384	94.09	92.38	92.60	85.79	78.02
HoCP 85-845	92.23	92.1	91.17	88.75	73.23
HoCP 91-555	89.62	85.04	88.88	80.16	65.22

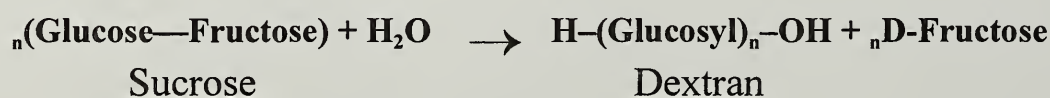
Table 2b. Fructose Concentrations

Cane Variety	% FRUCTOSE (on Brix Basis)				
	0 days	7 days	14 days	22 days	30 days
CP 70-321	0.80	0.86	0.86	1.17	4.05
CP 79-318	1.40	1.66	1.57	2.75	4.01
LHo 83-153	0.81	0.88	0.79	1.12	3.11
LCP 85-384	0.80	0.91	0.87	1.41	2.64
HoCP 85-845	0.93	0.91	0.97	1.92	3.66
HoCP 91-555	1.47	2.20	1.56	2.54	3.90

Table 2c. Glucose Concentrations

Cane Variety	% GLUCOSE (on Brix Basis)				
	0 days	7 days	14 days	22 days	30 days
CP 70-321	0.77	0.83	0.84	1.11	2.93
CP 79-318	1.34	1.66	1.49	2.72	5.64
LHo 83-153	0.84	0.88	0.76	1.00	2.47
LCP 85-384	0.83	0.80	0.73	1.10	2.26
HoCP 85-845	0.97	0.92	0.99	1.22	3.22
HoCP 91-555	1.55	2.12	1.61	2.37	3.90

Glucose/fructose (G/F) ratios were also calculated and are listed in Table 3. Lowering of G/F ratios usually indicates dextran formation because dextransucrase, an enzyme secreted extracellularly by *Leuconostoc* bacteria, hydrolyzes glucose from the sucrose molecule to form dextran, leaving fructose (from the sucrose molecule) as a secondary product (Legendre, *et al.*, 1985), which is illustrated in the following basic equation:



However, for the four varieties CP 79-318, LCP 85-384, HoCP 85-845, and HoCP 91-555, 30 days after the initial freeze, the G/F ratio actually increased, and in the case of CP 79-318 there was a dramatic increase (Table 3). This strongly suggests that dextran deterioration reactions were not solely responsible for the total freeze deterioration around this date, and that other, simultaneous

microbial infections and enzymic/chemical reactions were most likely occurring. These four varieties were obviously more susceptible to other microbial infections and deterioration reactions as well as infection from *Leuconostoc* bacteria. Furthermore, no significant correlations were found between G/F ratios and other deterioration criteria studied, which is most likely because G/F ratios are a reflection of multiple types of freeze induced cane deterioration.

Table 3. Variations in glucose/fructose ratios

Variety	Glucose/Fructose Ratios				
	0 days	7 days	14 days	22 days	30 days
CP 70-321	0.96	0.97	0.98	0.95	0.72
CP 79-318	0.96	1.00	0.95	0.99	1.41
LHo 83-153	1.04	1.00	0.96	0.89	0.79
LCP 85-384	1.04	0.88	0.84	0.78	0.86
HoCP 85-845	1.04	1.01	1.02	0.64	0.88
HoCP 91-555	1.05	0.96	1.03	0.93	1.00

Formation of Oligosaccharides, Mannitol and Ethanol in the Freeze Deteriorated Cane Varieties

Using factory cane juices which were allowed to deteriorate under different controlled conditions, Eggleston (2002) showed that the cane deterioration products oligosaccharides, mannitol and ethanol could be simultaneously analyzed using an IC-IPAD method with a sodium hydroxide/sodium acetate gradient. This method was similarly applied to the samples in this study. Typical chromatograms across the freeze dates for varieties CP 70-321 and HoCP 85-845 are shown in Figures 3 and 4, respectively.

Oligosaccharides. Iso-maltotriose, leucrose, palatinose, and 1-kestose oligosaccharides were analyzed in this study and marked varietal differences in formation of oligosaccharides were observed (see Figures 3-5).

Dextranase enzyme, secreted mostly by *Leuconostoc* bacteria, not only catalyzes the synthesis of dextran and dextran branch linkages using sucrose as the substrate, but in the presence of other carbohydrates named “acceptors” such as glucose, fructose, maltose, etc., also transfers glucose from the sucrose molecule to the carbohydrate. These are known as acceptor reactions and the products formed are acceptor products (Robyt and Eklund, 1982 and Robyt, 1995). Iso-maltotriose, leucrose, and palatinose are all such acceptor products (Robyt and Eklund, 1982) and, therefore, could be potential criteria for cane dextran deterioration. As illustrated in Figs. 5a and 5b, leucrose and palatinose were only measurable using the IC-IPAD method in this study, approximately 14 days after the first freeze, although leucrose was apparent in LCP 85-384 after 7 days. The detection of leucrose before palatinose is not surprising. Both leucrose and palatinose are acceptor products

formed when fructose is the acceptor, and their synthesis depends on the ring form of the fructose (Robyt, 1995). The major product is always leucrose, because it is formed from D-fructopyranose the most abundant ring form of fructose, whereas the minor product palatinose is formed when D-fructofuranose is the acceptor. Because of their relatively slow rate of formation by dextranase, both leucrose and palatinose only appear to be sensitive indicators of cane dextran deterioration when severe dextran formation has occurred, i.e., $> \sim 1500$ ppm/Brix of dextran. This is further evidenced by the fact that although 1058 ppm/Brix dextran formed after 7 days in LCP 85-384 neither leucrose nor palatinose were detected. Steinmetz, *et al.*, (1998) studying frost-damaged sugar beet found that the leucrose content was a direct indicator of dextran, although they found that mannitol was more strongly correlated with the quality of the damaged beets. In this study, the correlation between palatinose and dextran ($r^2 = 0.89$, $P < .0001$) was actually slightly greater than that for leucrose and dextran ($r^2 = 0.78$, $P < .0001$) although N values were only 12 because, in general, both of these oligosaccharides were not detected from 0-14 days. In comparison to leucrose and palatinose, the oligosaccharide iso-maltotriose was detected earlier, even after the first freeze at 0 days. This may be because iso-maltotriose is an acceptor product which is formed earlier than leucrose and palatinose by dextranase (Robyt and Eklund, 1982). Furthermore, there was not only a very strong correlation between isomaltotriose with dextran ($r^2 = 0.89$, $P < .0001$), but also mannitol ($r^2 = 0.91$, $P < .0001$) and pH ($r^2 = 0.91$, $P < .0001$) which strongly indicates that iso-maltotriose is the most sensitive oligosaccharide indicator for dextran cane deterioration.

The oligosaccharide 1-kestose is not associated with the formation of dextran by dextranase. It is formed from the action of other enzymes, including invertases (β -fructofuranosidases). Invertases not only hydrolyze sucrose into its constituent monosaccharides, glucose and fructose but if the sucrose concentrations are high enough (> 0.29 M sucrose) they are capable of catalyzing transfructosylating reactions with sucrose to produce various kestose (fructosyl sucrose) oligosaccharides of which 1-kestose is the most predominant (Pollack and Cairns, 1991 and Farine, *et al.*, 2001). Invertases occur in several isoforms in cane and are also produced by yeasts. It may also be possible that kestoses are also catalyzed by trans-fructosylating enzymes in the cane plant. A slight amount of kestoses can be formed in very acidic conditions by the acid degradation of sucrose (Eggleston, 2002) but this usually occurs at elevated temperatures. Except for CP 70-321, there were slight increases in 1-kestose concentrations for the varieties at 7 days after the initial freeze (see Fig. 5d). This is in contrast to the other cane deterioration criteria studied including dextran which, 7 days after the initial freeze increased in only two varieties: LCP 85-384 and HoCP 91-555 (Figure 2), although slight increases in isomaltotriose concentrations were seen in two other varieties (Figure 5c). This strongly suggests that although little microbial deterioration occurred after the first freeze, some enzymic cane deterioration occurred in all varieties except CP 70-321. Further evidence of this is the apparent increases in glucose and fructose concentrations (Table 2) for most varieties. For all varieties, 1-kestose concentrations became stable between days 7-14 days after the initial freeze but, except for CP 70-321, increased rapidly thereafter (Figure 5d). The very stable concentrations of 1-kestose from 0 to 30 days for CP 70-321 strongly indicates that CP 70-321 was very resistant to enzymic cane deterioration. This resistance may be because of a biochemical factor or because of a physical factor which protects the integrity of cells and their enzyme contents from freezes.

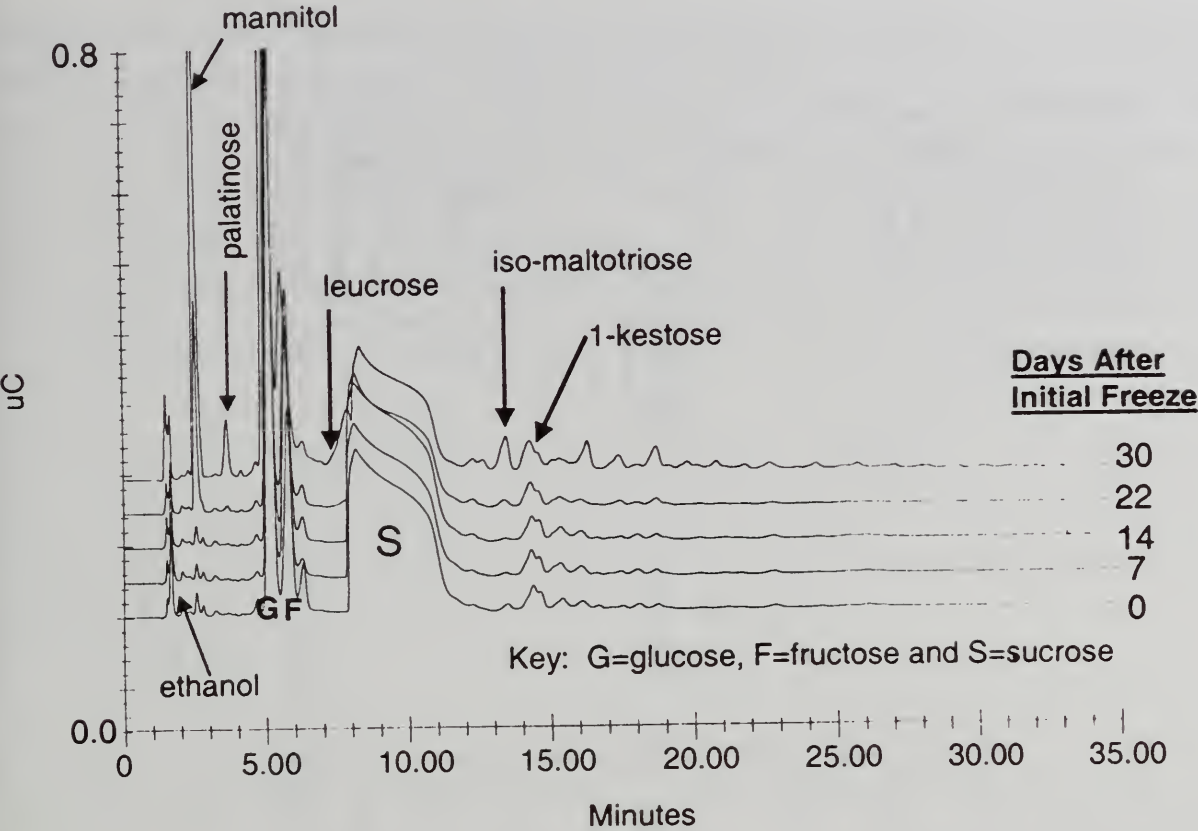


Figure 3. IC-PAD chromatograms for cane variety CP 70-321. (Brix standardized to 16.7.)

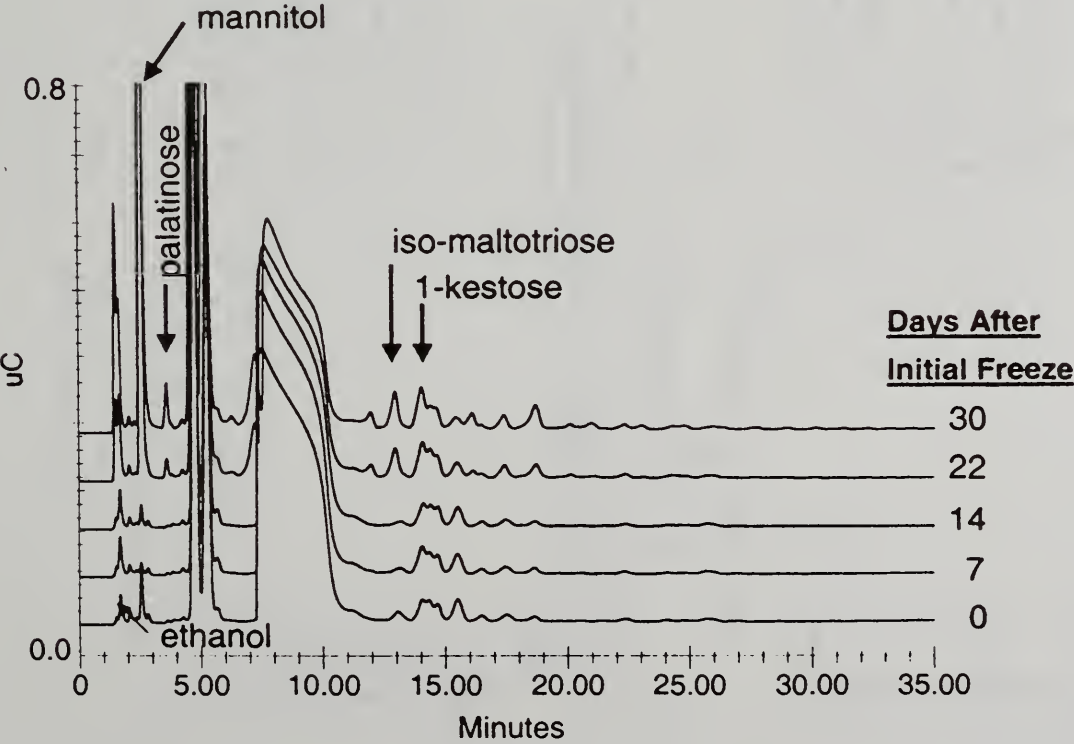


Figure 4. IC-PAD chromatograms of variety HoCP 85-845. (Brix standardized to 16.1.)

Fig. 5a. Changes in leucrose

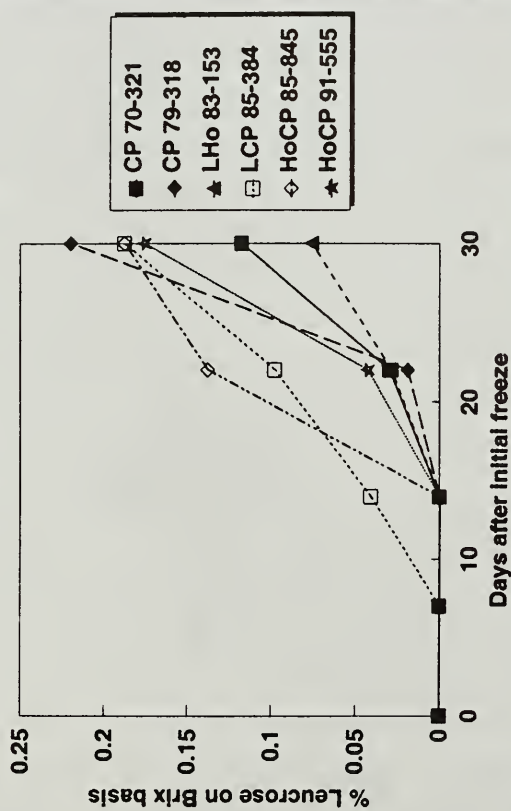


Fig. 5b. Changes in palatinose

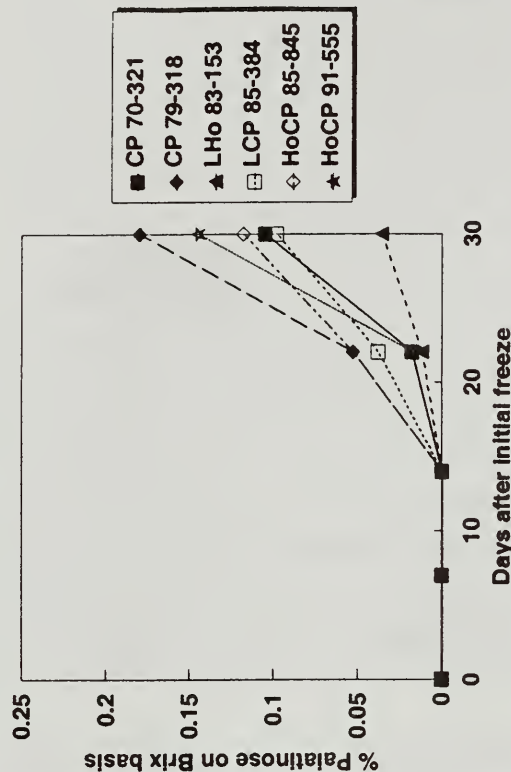


Fig. 5c. Changes in iso-maltotriose

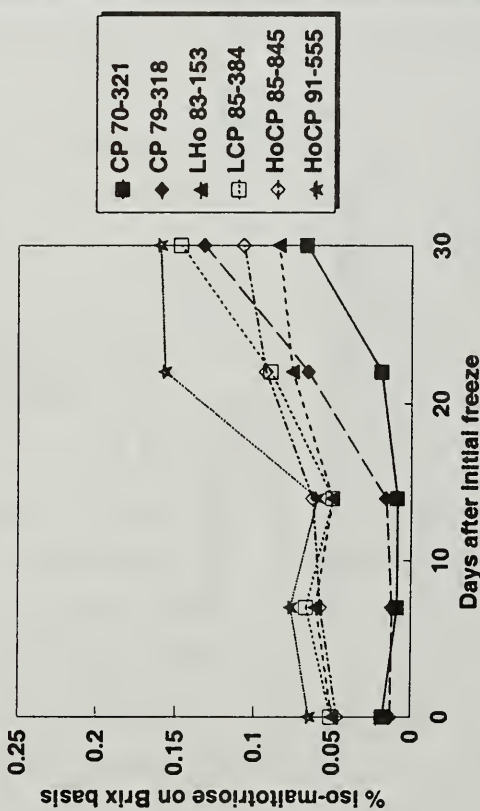
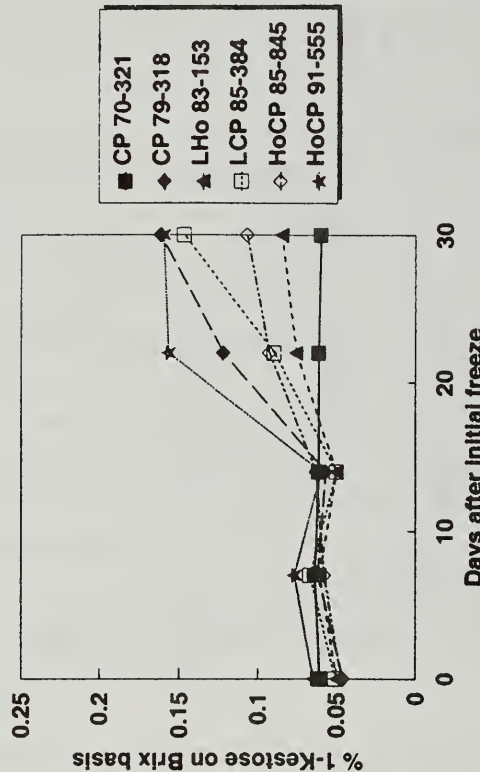
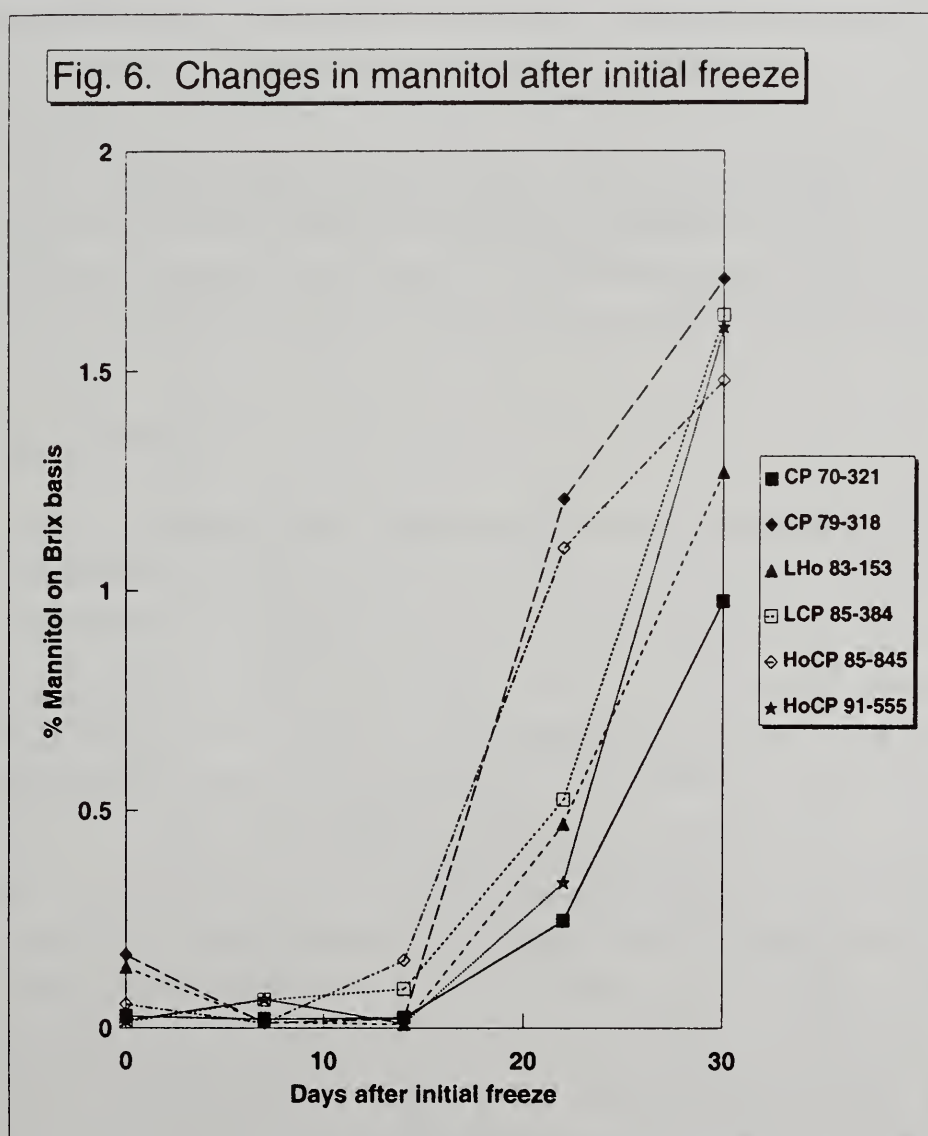


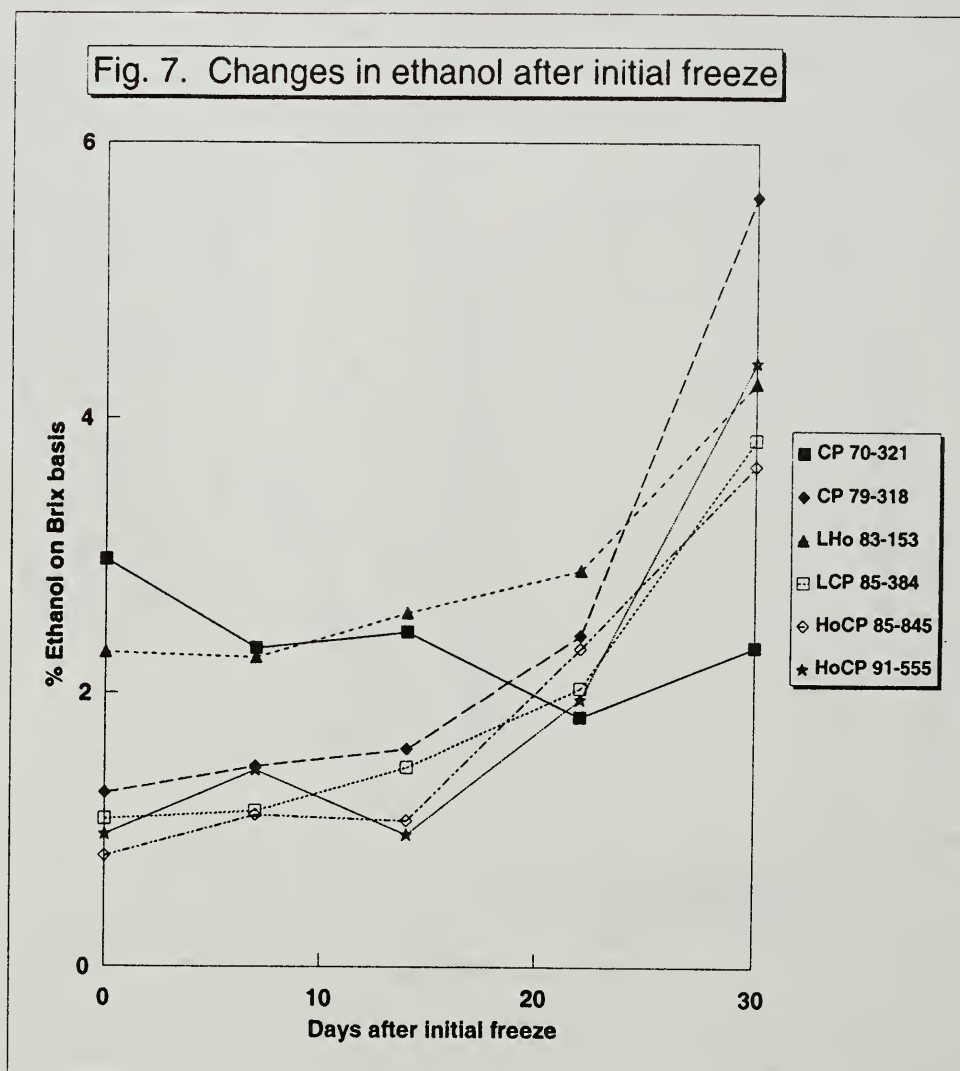
Fig. 5d. Changes in 1-kestose



Mannitol. Mannitol formation across all freeze dates is shown in Figure 6. For all varieties, a small amount of mannitol was present even after the first freeze at 0 days, whereas very little dextran polymer was detected (Figure 2). This may suggest that mannitol is an even better indicator of *Leuconostoc* growth in cane than dextran. Seven days after the initial freeze, similar to the dextran results (Figure 2) mannitol only formed in LCP 85-384 and HoCP 91-555, further indicating that these two varieties were the least resistant to *Leuconostoc* invasion even when no stalk cracks were visible. Dramatic varietal increases in mannitol occurred particularly after 22 and 30 days and at much higher concentrations than the oligosaccharides. Mannitol formation was least in CP 70-321 and worst in CP 79-318. Furthermore, there was a very strong correlation between mannitol and dextran ($r^2 = 0.84$, $P < .0001$) and between mannitol and isomaltotriose ($r^2 = 0.91$, $P < .0001$). However, the large advantage mannitol has over iso-maltotriose as a cane deterioration indicator, is that much higher concentrations are formed which can also be more readily detected by both IC-IPAD and GC techniques, and mannitol is known to directly impact cane processing (Bliss, 1975). Further studies will now be undertaken to ascertain the critical concentrations of mannitol that affect cane processing parameters such as viscosity or filterability.



Ethanol. Ethanol has been used as a cane deterioration indicator, particularly in burnt whole-stalk cane in South Africa (Lionnet & Pillay, 1987). Ethanol is a metabolic by-product of numerous yeast, bacterial and fungal reactions, and the amount formed depends on the type of microbe, as well as microbial growth parameters. Ethanol is especially a major by-product of yeast fermentation reactions, with yeast converting sucrose into ethanol and carbon dioxide, especially under dry and anaerobic conditions. *Leuconostoc* bacteria, besides forming dextran, can also produce ethanol although this is usually only the case if glucose, not sucrose, is the carbohydrate energy source (see Eggleston, 2002). Ethanol concentrations are illustrated in Figure 7. Like iso-maltotriose and mannitol, ethanol was present in the cane even after the first freeze at 0 days. This may also be a reflection of the maturity of the cane, previous yeast growth on the surface of the cane or previous animal or pest deterioration of the cane. For all varieties ethanol concentrations were relatively stable from 0-14 days and even decreased in CP 70-321 which is most likely because of evaporation. Except for CP 70-321, after 14 days ethanol increases were observed in all varieties strongly indicating that considerable microbial cane deterioration had occurred. However, in comparison to mannitol, ethanol was only weakly correlated ($r^2=.55$, $P<.001$) with dextran and did not always predict cane dextran deterioration which was the case with CP 70-321 (compare Figures 2 and 7). Eggleston (2002) similarly observed that ethanol was not always a direct indicator of cane dextran deterioration.



Significant Correlations Amongst Cane Freeze Deterioration Criteria

Significant ($P < .01$) Pearson r^2 correlation co-efficients greater than 0.50 are listed in Table 4. Although, as expected, sucrose was strongly correlated with its primary degradation products glucose and fructose, only moderate correlations were found with other deterioration criteria. This is because sucrose losses are due to a complex of deterioration reactions, mainly enzymic and microbial in nature, rather than one principal reaction mechanism. Fructose, palatinose, leucrose, and iso-maltotriose are all by-products from dextranase activity and, therefore, as expected were strongly correlated with dextran formation, particularly palatinose and iso-maltotriose (Table 4). *Leuconostoc* bacteria, as well as producing dextranase enzyme, also produces mannitol dehydrogenase an enzyme that catalyzes the formation of mannitol from fructose. Mannitol was observed to be strongly correlated with dextran ($r^2 = 0.84$, $P < .0001$), isomaltotriose ($r^2 = 0.91$, $P < .0001$) and pH ($r^2 = 0.92$, $P < .0001$) and confirmed previous observations by Eggleston (2002) that mannitol is a valid indicator of cane dextran deterioration. 1-Kestose was only moderately correlated with other freeze deterioration criteria (Table 4). This confirms that enzymic deterioration is only a minor contributor to total freeze-deterioration. In previous studies, Irvine and Legendre (1985) stated that there was a close relationship between post-freeze deterioration of sugarcane varieties and apparent sucrose, pH, titratable acidity, and dextran content of juice following a "hard" freeze (min. temperature 12.9° F) where all stalk tissue was damaged. Juice pH is not usually considered a sensitive indicator of post-freeze deterioration following a freeze of less severity (temperature no lower than 21.9° F); however, in this study, where all stalk tissue was damaged, pH was strongly correlated with dextran, mannitol and iso-maltotriose (all indicators of cane dextran deterioration) and to a lesser extent with sucrose, glucose, fructose, palatinose, and leucrose. Therefore, pH may be, in fact, more useful than originally thought as a post-freeze indicator of deterioration especially when all internal stalk tissue is damaged.

MAIN CONCLUSIONS

Marked differences were observed for most criteria for all varieties, particularly 22 and 30 days after the first freeze. Mannitol was strongly correlated ($r^2 = 0.84$) with dextran confirming its use as an indicator for cane dextran or *Leuconostoc* deterioration. Because it is a low molecular weight (MW) compound it is much easier to detect than the high MW polysaccharide, dextran. In comparison, ethanol was only weakly correlated ($r^2 = 0.55$) with dextran and did not always predict cane dextran deterioration. Iso-maltotriose was a better oligosaccharide indicator of freeze deterioration than both leucrose and palatinose and was strongly correlated ($r^2 = 0.89$) with dextran. pH was a strong indicator of dextran ($r^2 = -0.85$), mannitol ($r^2 = -0.92$) and isomaltotriose ($r^2 = -0.83$) formation. Four of the varieties, CP 79-318, LCP 85-384, HoCP 85-845 and HoCP 91-555, were shown to be susceptible to other sources of microbial and enzymic deterioration as well as dextran deterioration from *Leuconostoc* bacteria, especially 30 days after the first freeze. This was indicated by increased glucose/fructose ratios, ethanol formation, changes in 1-kestose concentrations, and further sucrose losses. Legendre, *et al.*, (2001) using previously used cold tolerance criteria such as theoretical recoverable sucrose (TRS), generally ranked the cane varieties, from best to worst, as follows: CP 70-321, LHo 83-153, LCP 85-384, HoCP 85-845, HoCP 91-555, and CP 79-318. However, with the increased sensitivity of mannitol and oligosaccharides, general ranking was different with, from best to worst: CP 70-321, LHo 83-153, HoCP 85-845, LCP 85-384, HoCP 91-555, and CP 79-318, with very little difference in the ranking of the latter three varieties.

Table 4. Significant correlations of freeze deterioration criteria^a

	Sucrose	Glucose	Fructose	G/F	Dextran	Ethanol	Mannitol	pH	Brix	Palatinose	Leucrose	isomalto- triose	1- Kestose
Sucrose	--												
Glucose	-0.95 ^b .0001 ^c	--											
Fructose	-0.85 .0001	0.86 .0001	--										
G/F				--									
Dextran	-0.76 .0001	0.67 .0001	0.79 .0001		--								
Ethanol	-0.64 .0001				0.55 .0001	--							
Mannitol	-0.73 .0001	0.63 .0001	0.72 .0001		0.84 .0001	0.71 .0001	--						
pH	0.75 .0001	-0.75 .0001	-0.78 .0001		-0.85 .0001	-0.67 .0001	-0.92 .0001	--					
Brix			-0.52 .0001						--				
Palatinose	-0.72 .0005	0.67 .0011	0.61 .0027		0.89 .0001	0.57 .0045	0.73 .0004	-0.61 .0027		--			
Leucrose					0.78 .0001	0.57 .0042	0.62 .0025	-0.53 .008		0.79 .002	--		
isomalto- triose	-0.78 .0001	0.68 .0001	0.70 .0001		0.89 .0001	0.62 .0001	0.91 .0001	-0.83 .0001		0.87 .0001	0.82 .0001	--	
1- Kestose	-0.67 .0001	0.60 .0001	0.54 .0001		0.52 .0001		0.62 .0001					0.67 .0001	--

^a Only significant (P<.05) correlations greater than r²=0.50 are listed, ^b The r² value, ^c The probability P level

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HIGH MOLECULAR WEIGHT COMPONENTS IN BEET PROCESSING: CHANGES THAT OCCUR IN PROCESS

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ABSTRACT

Literature has shown the importance of the high molecular weight components in color formation and sugar quality, for both cane and beet processing. In this study, the high molecular weight (HMW) components in beet processing, including colorants and polysaccharides, were followed throughout beet processing stages at one U.S. factory during the 2000-2001 season. The object of the study was to determine the composition of the HMW in beet processing and to note changes that occurred during processing. Gel permeation chromatography (GPC) was used to observe the HMW. Results showed that raw juice entering the factory contained several very high molecular weight peaks, probably pectin-like polysaccharides, which were removed or destroyed during clarification. Subsequently, a series of colorants formed during clarification, the pattern of which remained largely unchanged throughout the process. These HMW materials were associated with polysaccharides. A subset of these colorants continued into the crystalline white sugar.

A composite sample of thin and thick juice across the season was separated on strong base styrenic resin (IRA 900 Cl) to obtain two fractions, one enriched in HMW colorant and one enriched in HMW polysaccharide. The composition of these fractions is briefly described.

INTRODUCTION

Courtois (2001) stated in a presentation, that sugar is “an affair of macromolecules,” referring to the importance of high molecular weight molecules in sugar processing and in color transfer from syrup to sugar crystals. Beet sugar colorant has been studied for over a century (Broughton, *et al.*, 1987), with emphasis on color precursors, the role of enzymes on phenolic compounds (Gross and Coombs, 1976; Winstrom-Olsen, 1981; Winstrom-Olsen, *et al.*, 1979; Maurandi, 1988; Rhinefeld, *et al.*, 1984), the development of color on storage (Shore, *et al.*, 1984; Richmond, 1990; Godshall, *et al.*, 1991; 1998a), and the distribution of color within the crystal (Shore, *et al.*, 1984; Mantovani, *et al.*, 1986). In recent years, a great deal of interest has developed in comparing the nature of cane and beet

colorants to understand the differences between the two types in transfer of color into crystals (Godshall and Baunsgaard, 2000). New techniques, such as fluorescence spectroscopy, have been employed to study beet colorants (Baunsgaard, *et al.*, 2000; 2001).

It is generally accepted in the literature that high molecular weight compounds have a greater tendency to go preferentially into the sugar crystal and thus impact refined sugar quality (Tu, *et al.*, 1977; Godshall, *et al.*, 1987; Lindeman and O'Shea, 2001). Reports in the literature also indicate that cane sugar colorants have a considerably higher molecular weight than beet sugar colorant. The molecular weight range of beet sugar colorants has been reported as 5000 to 40,000 Da (Broughton, *et al.*, 1986), whereas cane sugar colorants are in the 30,000 to 1,000,000 Da range (Godshall, *et al.*, 1988).

The role of polysaccharides in beet processing has not been adequately addressed. Godshall and Baunsgaard (2000) noted that polysaccharides in processing tend to be lower over-all than in cane, and may account in part for the lower color transfer rate of beet colorant into crystal compared to cane colorant. Cane polysaccharides have been shown to be associated with polyphenolic acids and are involved in color formation in cane juice (Godshall, *et al.*, 1998b; 2001). Fares, *et al.*, (2001) reported that the presence of monomers of galacturonic acid in factory juice in beet processing increased color formation. Godshall showed the presence of two high molecular weight compounds in beet white sugar, one, about 20,000 Da, having a yellow color and polysaccharide nature (Godshall, 1992).

As part of SPRI's continuing study of the nature of beet and cane colorants, this study was undertaken to follow the progress of the high molecular weight (HMW) materials throughout beet processing stages across the 2000-2001 season. Samples were provided by a SPRI sponsoring company from the United States.

The objective of the study was to determine the composition of the HMW material in beet processing and to note changes occurring during processing.

MATERIALS AND METHODS

Samples were obtained from a sponsoring beet company in the western United States and taken from beet processing stages across the 2000-2001 season. Dates of sample collection were: 11/8/00; 11/9/00; 12/23/00; 12/24/00; 1/9/01; 1/10/01; 2/26/01; 2/27/01.

Samples collected were: Raw juice, first carbonation juice, thin juice, softener supply, thick juice, standard liquor, white sugar and molasses.

To obtain high molecular weight (HMW) material representative of the season, three sets of samples, corresponding to the beginning (BPS #1, 11/8/00), middle (BPS #3, 12/23/00), and end (BPS #8, 2/27/01) of the season were dialyzed in cellulose acetate dialysis tubes, having a nominal molecular weight cut-off of 12,000 Da. The obtained HMW nondialyzable material (the tenate) was evaporated to a small volume and freeze dried.

Molecular weight determinations of the tenates were carried out by gel permeation chromatography (GPC) by HPLC using five GPC TSK diol columns in series (TSK Gel PWXL, 6000, 5000, 4000, 3000 and 2500 angstrom pore size), using 0.1 N NaCl/10% acetonitrile as the eluent at 0.6 ml/min (Vercellotti, *et al.*, 1996). Detection of peaks was accomplished by UV detection at 270 nm and refractive index (RI) detection in series. Selected samples were also analyzed by the Folin-Ciocalteu reagent for polyphenol/protein reactive material (Lowry, *et al.*, 1951), for pectin by m-hydroxydiphenyl after sulfuric acid-borate treatment (Kintner and Van Buren, 1982), and hydrolyzed with trifluoroacetic acid to determine the composition of the monomer sugars as alditol acetate esters by gas chromatography.

Tenate fractions from BPS #1 were also separated using C-18 reverse phase adsorption on J.T. Baker octadecyl reverse phase bulk packing into aqueous, methanol and methylsulfoxide soluble fractions.

Color and total polysaccharides were determined for raw juice, thin juice, thick juice, standard liquor, white sugar and molasses, using ICUMSA and SPRI methods.

Subsequently, the remaining thin juice, thick juice and standard liquor from across the season was composited to obtain a representative seasonal sample of the colorants. Aliquots of this composite were passed through a column containing about 200 ml of strong anionic resin IRA-900 in the chloride form. Product recovered with water was called the "eluate." The resin was regenerated with strong salt and base to obtain a fraction called the "regenerate." The composite (feed to the resin), eluate (product) and regenerate were analyzed for color and polysaccharide and were dialyzed to obtain the HMW for GPC and further analyses.

RESULTS AND DISCUSSION

Polysaccharides. Polysaccharide concentration in the process samples is shown in Table 1 and Figure 1. The presence of a precipitate in the 1st carbonated juice and the softener supply interfered with the determination of polysaccharides, and they could not be correctly calculated.

Table 1. Total polysaccharides in beet processing across the season (ppm on solids)

Sample	11-8-00	11-9-00	12-23-00	12-24-00	1-9-01	1-10-01	2-26-01	2-27-01
Raw juice	3934	3816	3068	3342	3153	4011	5131	6080
Thin juice	1168	740	848	861	798	901	1534	1542
Thick	930	752	594	651	625	727	1678	1502
Std. liquor	876	652	402	724	491	620	1059	1153
Sugar	18	84	49	129	84	105	51	94
Molasses	4992	4536	4442	4839	4076	3764	4868	4622

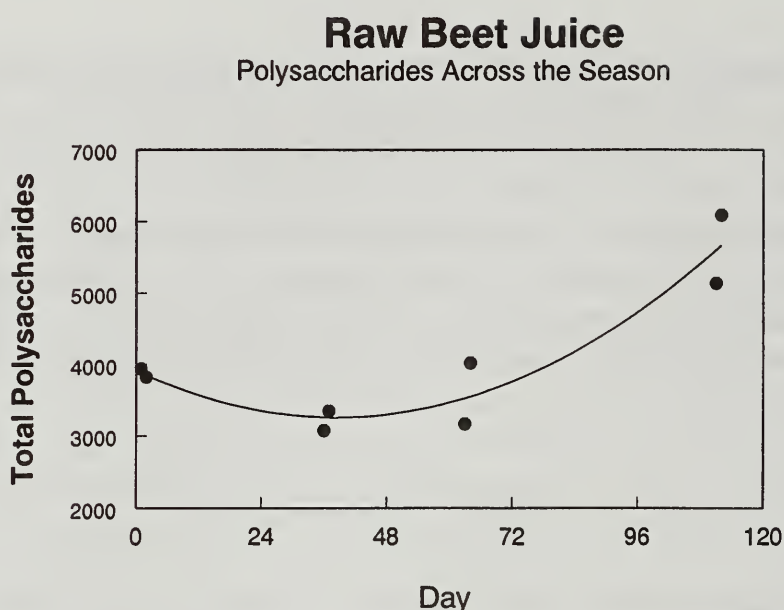


Figure 1. Changes in polysaccharide concentration in raw beet juice across the season.

Polysaccharides increased toward the end of the season. Averaging the two dates within each month, we note that the polysaccharide concentration is about the same through November, December and January, but increased by about 45% in February, as shown below. By late February, the beets have been in long term outside storage, some ventilated and some unventilated, conditions which may lead to the formation of stress related compounds, such as polysaccharides, phenolics and oligosaccharides.

Average total polysaccharides in raw juice by month

November 8/9	3875 ppm
December 23/24	3205
January 9/10	3582
February 26/27	5606

A significant portion of polysaccharide (77%) was removed during clarification, reflecting the removal and/or destruction of pectin and other soluble cell wall polysaccharides in the beet juice. (This is later confirmed in the results for the dialyzed material in a later section.) The seasonal averages for total soluble polysaccharide in the various processes are shown below.

Average total polysaccharides by process across the season

Raw juice	4067 ppm	
Thin juice	918	(77.4% elimination)
Thick juice	932	
Standard liquor	747*	
White sugar	77	(>90% elimination)
Molasses	4518	

* Different amounts of standard liquor polysaccharides reflect differing inputs from other streams that constitute the standard liquor.

Color. The results for color in the process samples are shown in Table 2. Color in the raw juice increased linearly across the season (Figure 2). Averaging the two dates within each month, we see this trend in raw juice color:

Raw juice color average for each month

November 8/9	877
December 23/24	1303
January 9/10	1289
February 26/27	2151

Table 2. Color in beet processing across the season

Sample	11-8-00	11-9-00	12-23-00	12-24-00	1-9-01	1-10-01	2-26-01	2-27-01
Raw juice	858	895	995	1611	1455	1123	1988	2314
1 st Carb	939	1828	1628	2238	2472	2153	2787	3117
Thin juice	1466	751	988	1164	1332	1385	2065	1831
Thick	1056	940	1263	1467	1817	1778	2671	2088
Std. liquor	1509	1189	1411	1696	1826	1635	2217	1974
Sugar	18	18	22	24	22	29	24	25
Molasses	28,371	30,796	37,922	36,269	34,240	36,481	46,806	45,418

The average color across the season for each processing stage is shown below:

Average color across the season for process stages

Raw juice	1405
1 st Carb	2145
Thin juice	1373
Thick juice	1635
Std liquor	1682
White sugar	23
Molasses	37,038

A 145% increase in color in thin juice occurred across the season. As expected, there was an increase in color in 1st carbonation juice, reflecting the alkaline destruction of invert, but after clarification, the color was not too different in thin juice compared to raw juice (mean of 1405 IU in raw juice vs. 1373 IU in thin juice). Thick juice color was higher across the season than thin juice color, with the anomalous exception of the Nov. 8th date (mean of 1373 ICU in thin juice and 1635 in thick juice, a 19% increase), representing color formation in the evaporators. There was very little change in color from thick juice to standard liquor (1635 IU in thick juice and 1682 in standard liquor). The molasses color reflects the upward trend, going from an average color of 29,584 in November to 46,112 in February, a 55.9% color increase. The ratio of standard liquor to white sugar color was 73:1.

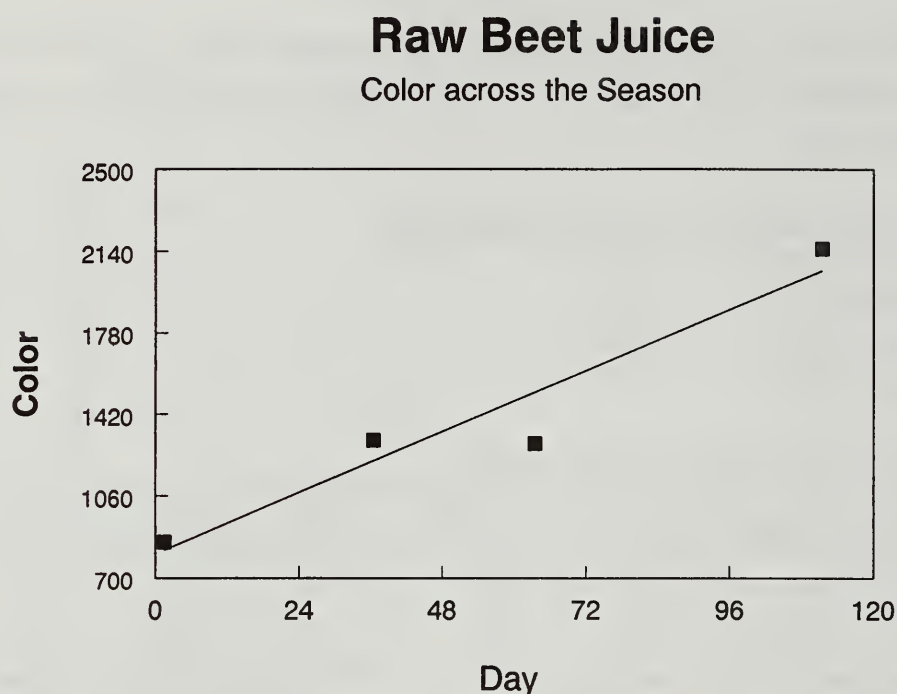


Figure 2. Changes in the color of raw beet juice across the season.

Chemical Composition of Tenates

Table 3 presents data on the amount of nondialyzable material (tenate) present in the beet processing samples across the season. Table 4 presents data on the proportion of carbohydrate (ie, polysaccharide), pectin, and Folin-Ciocalteu reactive substance in each tenate from samples taken November 8, 2000.

Table 3. HMW components (>12,000 Da) in beet processing across the 2000/2001 season

Sample	ppm on solids		
	11/8/00	12/23/00	1/27/01
Raw juice	4580	15,340	8775
Thin juice	3490	2423	2952
Thick juice	2024	1838	2270
Standard liquor	1497	1201	1723
White	77	107	141
Molasses	11,088	9789	6860

Table 4. Composition of the nondialyzable material (tenate) in beet processing. Samples were taken Nov 8, 2000.

Sample	Appearance of Tenate	% CHO by $\phi\text{H}_2\text{SO}_4$	% Folin-Ciocalteu reactive	% Pectin
Raw Juice	White	85.9	2.6	27.2
Thin Juice	Off-white	33.5	8.7	3.9
Thick Juice	Off-white	45.9	20.3	5.2
Std. Liquor	Light tan	21.8	18.3	4.1
White Sugar	Off-white	23.4	—*	—*
Molasses	Brown	45.0	38.9	4.9

* Not enough sample to analyze.

CHO = carbohydrate; a measure of the amount of polysaccharide in the tenate; measured by the phenol-sulfuric acid method.

The majority of the pectin in raw juice was removed at the clarification step, and a uniform quantity remained throughout the rest of the process. While the Folin-Ciocalteu test is usually considered a test for protein, this reagent also reacts with polyphenolic compounds. Since it is not possible that protein is being made in thin juice and in thick juice, the results shown in Table 4 represent the changes that occurred in the high molecular weight material during carbonation and evaporation, as colorant was being formed.

The tenates appear light colored (off-white to pale tan) when dry, but in solution have more visibly yellow to light brown color.

The carbohydrate content of the tenates represents polysaccharide, only a small portion of which is pectin. There was also a significant quantity of insoluble matter in the tenates, which we feel represents insoluble calcium salts.

Table 5 shows the monomer sugar composition of the polysaccharide portion of the tenates in the set of samples from November 8, 2000. The material was mainly arabinogalactan, with the composition remaining fairly uniform throughout the process. It should be noted again that the carbohydrate (ie, polysaccharide) portion of the HMW, except for the raw juice, was less than 50% of the HMW. The remainder is expected to be protein, colorant polymers and insoluble calcium salts.

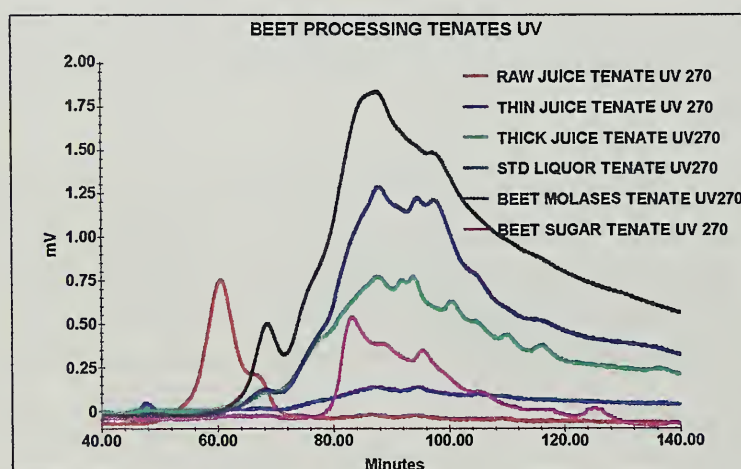
Table 5. Monomer composition of polysaccharide in tenates from Nov. 8, 2000.

Sample	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose
Raw juice	2.1	65.1	3.6	4.2	23.1	2.0
Thin juice	1.9	61.5	1.2	2.8	29.3	3.3
Thick juice	1.1	59.5	0.8	6.8	28.4	3.5
Std. liquor	1.1	62.4	0.8	5.0	27.7	3.2
White Sugar	2.2	48.6	1.5	7.6	27.4	10.3
Molasses	0.9	57.4	0.8	3.6	31.7	5.8

GPC of beet processing samples. Gel permeation chromatography (GPC) was performed on the three sets of tenates from the early, middle and late part of the season. Figure 3 shows the overlaid chromatograms of the tenates from the early season (November). While there are individual differences, the over-all pattern noted in Figure 3 is quite similar across the season. That is, the raw juice enters the factory with a set of very high molecular weight peaks, probably pectin-like polysaccharides, which are removed or destroyed during clarification. Colorant forms after clarification and builds up during evaporation. Similar colorants remain in the white sugar, with a bias toward the higher molecular weight end of the colorants.

The changes in the molecular weight of the various GPC peaks and their appearance is further emphasized visually in Figure 4a-c, which shows individual overlays of raw juice, standard liquor and white sugar across the season.

Beet Processing Colorant Development

**Figure 3.** Chromatograms of HMW in beet processing from November 8, 2000.

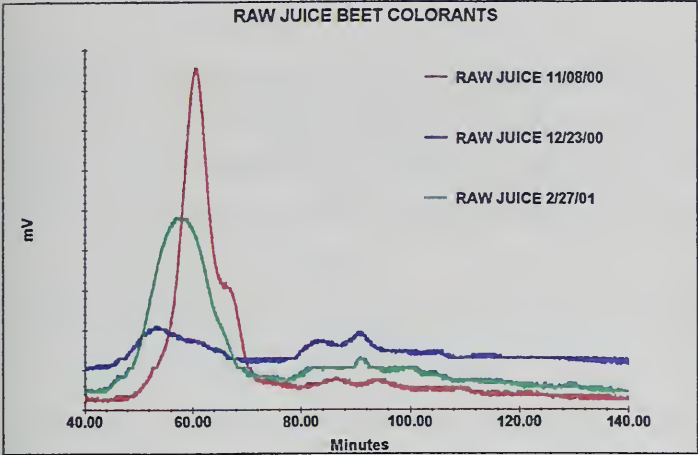


Figure 4a. Overlaid chromatograms of raw juice HMW across the season.

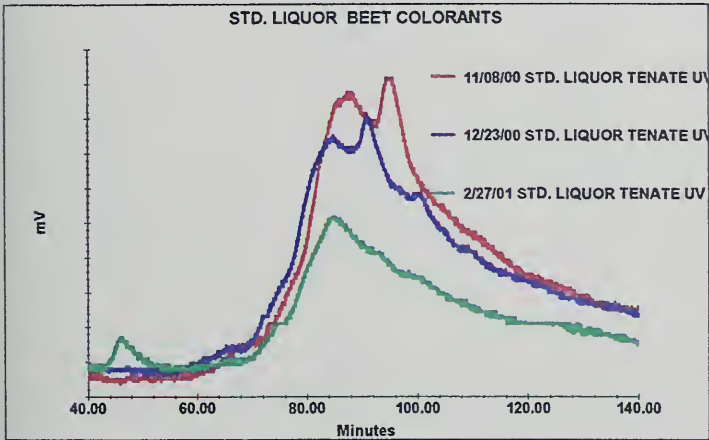


Figure 4b. Overlaid chromatograms of standard liquor HMW across the season.

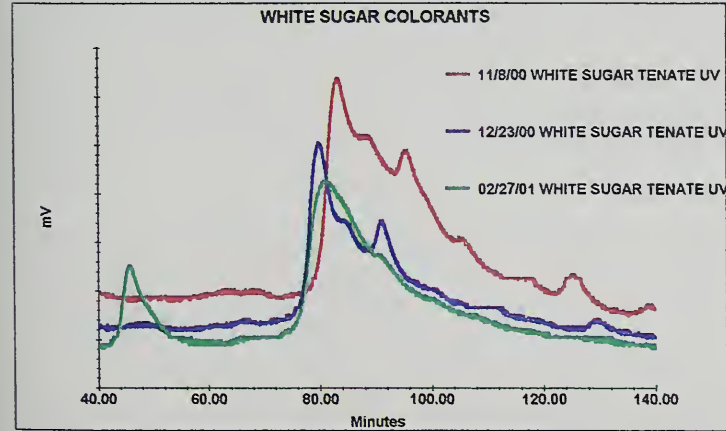


Figure 4c. Overlaid chromatograms of white sugar HMW across the season.

The molecular weight ranges of peaks for the first set (11/9/00) are given in Tables 6a-c. These data confirm the visual observation that raw juice has a higher molecular weight range (90% of peak area is >140,000 Da) than the rest of the process samples. The HMW is similar throughout the rest of the process, with differences being mainly in the proportion of peaks seen.

Table 6. Molecular weight of peaks in HMW material in beet processing. Detection = 270 nm

Raw Juice			Thin Juice		
Ret. time	MW	%	Ret. time	MW	%
52.4	537,637	5	47.4	924,291	7
57.2	323,122	21	57.5	313,630	4
60.5	230,874	51	67.3	118,981	6
66.5	141,165	18	77.2	48,573	10
86.1	22,792	2	87.0	21,222	18
91.8	14,471	2	94.3	11,993	19
126	1451	1	105	5558	13
			114	2913	9
			121	1892	8
			129	1176	6

Table 6b. Molecular weight of peaks in HMW material in beet processing. Detection = 270 nm

Thick Juice			Standard Liquor		
Ret. time	MW	%	Ret. time	MW	%
45.0	1,208,179	1	67.7	115,429	2
64.5	156,390	1	79.4	40,062	9
68.9	102,813	2	87.8	19,897	23
78.1	44,633	10	94.4	11,875	21
87.7	20,106	19	97.3	9583	21
93.6	12,633	19	103	6501	13
100	7726	15	113	3170	7
104	6039	11	126	1419	4
109	4121	9			
116	2696	7			
122	1861	4			
132	1013	2			

Table 6c. Molecular weight of peaks in HMW material in beet processing. Detection = 270 nm

White Sugar			Molasses		
Ret. time	MW	%	Ret. time	MW	%
51.4	599,297	1	68.6	105,745	7
61.2	216,353	1	78.1	44,633	12
83.3	28,823	33	82.5	30,816	20
87.0	21,171	25	87.5	20,334	25
95.1	11,295	22	95.9	10,609	19
104	6035	9	112	3513	10
113	3263	3	122	1791	7
125	1548	4			
138	725	2			

Although the HMW tenates represent material that is supposed to be >12,000 daltons, based on the cut-off of the dialysis bags, some peaks shown in Tables 6a-c have lower MW than 12,000. This has been observed before, and it is our feeling that this represents material that may have agglomerated during the very mild dialysis procedure and which was subsequently disassociated via shear forces upon subsequent treatment (ie, evaporation, freeze drying and chromatography).

Fractions obtained on C-18 columns. Several samples were fractionated on small C-18 columns using water, methanol and DMSO to extract colorant. The fractions were recovered, dried and weighed. In all cases, excellent recovery of the sample was obtained. An understanding of the chemistry of these fractions is still pending, but it is of interest to note here that DMSO extracted the most highly colored of the fractions while the aqueous fraction recovered the bulk of the material, but it had little color. Table 7 shows the amounts recovered from two of the white sugar samples and the appearance of each fraction.

Table 7. Fractionation of white sugar HMW tenates on C-18.

Sugar	Aqueous	% of wt	MeOH	% of wt	DMSO	% of wt	% recovery
11/8/00	White	62.3	Cream	20.8	Slightly yellow	15.6	99
2/27/01	Cream	62.2	Slightly yellow	18.3	Yellow-orange	17.1	98

HMW in Molasses.

When the chromatograms of molasses tenates using UV and RI detection were compared, it was noted that the RI-detected peaks were of a higher molecular weight than the UV-detected peak. Most peaks were detected by both methods, but the RI peaks in the higher MW range were more intense and represented a greater proportion of the total peak area at a higher MW. The major RI peak had a retention time of 57-73 min and averaged a MW of around 180,000 Da, representing 40-50% of total peak area. By contrast, no peak was detected by UV in that area of the chromatogram. Table 8 summarizes the peak MW and proportions for molasses tenates from November and December.

Table 8. MW of peaks separated by GPC of 2 molasses tenates, detected simultaneously by UV at 270 nm and RI.

Molasses tenate, 11/8/00				Molasses tenate, 12/23/00			
Ret. time	Det'n	MW	% polymers	Ret. time	Det'n	MW	% polymers
44-48	UV	993,465	0.2	44-49	UV	837,127	2.2
64-73	UV	99,465	5.6	60-68	UV	145,940	4.5
75-86	UV	31,798	26.7	71-78	UV	60,216	11.1
87-91	UV	17,800	19.2	80-89	UV	27,544	36.7
93-106	UV	8846	30.0	91-100	UV	11,704	21.4
108-122	UV	3030	14.5	102-116	UV	4619	19.6
125-140	UV	1215	3.8	120-138	UV	1360	6.4
47-51	RI	776,573	1.0	45-53	RI	837,127	2.3
58-73	RI	167,984	44.8	58-71	RI	192,758	40.5
74-80	RI	56,146	21.5	72-82	RI	63,447	18.6
84-91	RI	24,000	23.0	83-88	RI	28,065	18.4
93-96	RI	11,895	2.3	89-93	RI	15,645	7.5
97-112	RI	5219	7.4	105-127	RI	2932	8.39

This is interpreted to mean that peaks detected by RI have a largely carbohydrate character and represent polysaccharides. Peaks detected by UV represent aromatic and conjugated species and are interpreted to be colorant-type constituents or precursors. When peaks are detected by both RI and UV, two possibilities exist -- these are peaks with carbohydrate and colorant properties (such as observed in cane sugar colorants) or they may be separate species with similar retention times.

The clearly delineated difference in beet molasses between very high MW RI/polysaccharide peaks and lower MW UV/colorant peaks helps to explain why it is easy in chromatographic separation of beet molasses for sugar extraction to end up with a very highly colored sugar-containing extract with little polysaccharide, thereby facilitating sugar recovery.

Composite Sample Separation on Resin.

The composite sample described in Materials and Methods was passed through a column of the strong anion exchange resin, IRA 900, in the chloride form. The purpose for doing this was to determine if colorant and polysaccharide could be separated. The composition is shown below.

Composition of composite sample

Ash	2.75%
Calcium	0.008%
Purity	91.02% (sucrose by HPLC)
Glucose	0.160%
Fructose	0.173%
Color	1679
Polysacch.	1403 ppm
HMW	2033 ppm (the nondialyzable material >12,000 Da)

The resin provided a clean separation of color from sugar, but not of the polysaccharide, about 40% of which remained in the sugar-containing eluate. (89.2% of color was removed by the resin, and 60.1% of the total polysaccharide was removed.) The tenate of the eluate was a pure white material, which on GPC was shown to be a fairly homogeneous peak of about 30,000 Da. The tenate of the regenerate was brown in color, and GPC showed a range of peaks starting at about 20,000 Da and going to lower molecular weights. The overlaid chromatograms are shown in Figure 5.

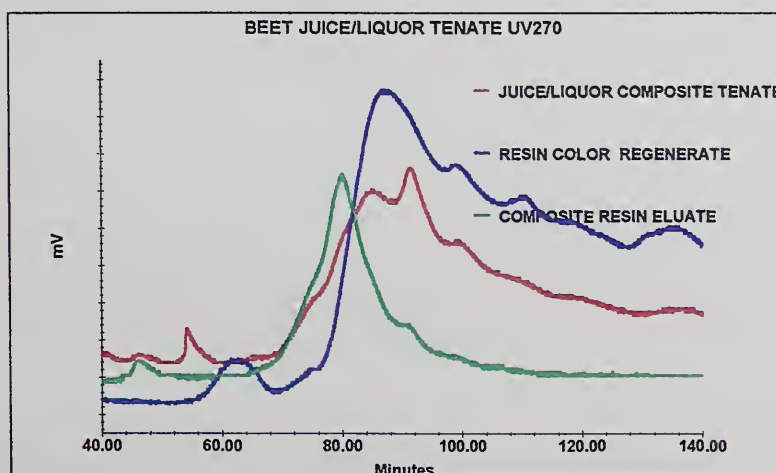


Figure 5. Overlaid chromatograms of HMW from beet composite sample (red), resin regenerate (blue) and eluate (green).

A UV/visible scan of the tenates showed that much of the UV-active material had been removed by the resin, so that little color was to be found in the eluate, and most of it was recovered in the regenerate.

The composition of the HMW in the resin fractions and the composite are shown in Table 9.

Table 9. Composition of HMW in beet composite sample and fractions obtained.

Property	Composite (feed)	Eluate (product)	Regeneate (waste)
Tenate, ppm	2033	621	692
Carbohydrate, %	46.5	61.9	25.1
Pectin, %	17.1	22.5	13.0
Phenol biuret, %	27.9	15.2	39.2
Rhamnose, %CHO	1.85	1.69	2.87
Arabinose, %CHO	43.66	45.20	42.38
Xylose, %CHO	2.23	1.01	4.30
Mannose, %CHO	4.45	7.70	4.12
Galactose, %CHO	30.77	30.82	39.39
Glucose, %CHO	17.03	13.58	6.95

The data in Table 9 show that about half of the HMW is removed by the resin, and the material removed is of a different composition. It is much lower in carbohydrate (ie, polysaccharide) than the eluate (25.1% vs 61.9%), and it contains the bulk of the colorant, which is of a lower molecular weight than the eluate material. The regenerate polysaccharide is relatively higher in rhamnose, xylose and galactose than the eluate polysaccharide and much lower in glucose and mannose.

The RI traces of the three tenates show higher molecular weight species in the HMW which are not UV-active, indicating the possibility of pure polysaccharide not associated with colorant. The large UV-active peak in the eluate, at about 80 min is also RI-visible, indicating some polysaccharide associated with phenolics. However, this particular peak has no visible color; it may be of a pectinaceous nature.

The GPC data from this resin experiment would indicate that most of the polysaccharide in beet processing samples is not associated with the colorant that forms in process.

SUMMARY

This study has shown the changes that occur in color, polysaccharide and high molecular weight constituents that occurred across the 2000/2001 season in a Western USA beet factory. The climatic conditions could be considered normal, and no unusual processing problems arose. The profile of HMW constituents, taken during the early, middle and later part of the season, showed individual differences, but an over-all similar pattern: Very HMW material, probably pectinaceous in nature, came in with the raw juice. There was little or no evident HMW colorant in the raw juice. The very high molecular weight pectinaceous material in the raw juice was partially destroyed or removed during carbonation/clarification, and there was subsequent formation of colorant in the thin juice that increased about 20% upon evaporation to make thick juice.

Some polysaccharide continued through process, but this was of a lower molecular weight profile than the material originally in the raw juice.

The colorant formed on carbonation appears to be the same colorant that carried into the crystal, with a bias to higher molecular weight components.

The HMW material that is transferred into the sugar crystal is responsible for some, probably a majority, of the white sugar color. This material occurs as a yellow solution, and upon freeze drying, was an off-white to light brown fluffy material. In previous studies, it has been observed to darken on standing (unpublished SPRI results). Fractionation of this material on octadecyl columns (C18) showed different solubilities in water, methanol and DMSO, along with different colors and GPC patterns (not reported here). An examination of the chemistry of these fractions is ongoing.

The resin experiment on a composite sample indicates that the polysaccharide and colorant are separate throughout the process. Earlier work has, however, indicated that lower polysaccharide content is preferable for lowering the transfer of color into crystals.

The resin work has also shown the strong affinity anionic resin has for the beet sugar colorant that is formed in process. Since this colorant goes into the sugar crystal, and also possibly is found in the film around the crystal, and it seems to be involved in color build-up on storage, ways to remove this colorant should be explored. In ongoing work (not reported here) we have noted that this color, while not very dark, appears to be highly autocatalytic: That is, the more color that is present at the start, the more color that is likely to form over time.

The HMW colorant in molasses was shown to be separated from a set of very HMW peaks detected by RI, probably polysaccharide in nature, helping to explain why a very highly colored molasses separator extract can produce low color white sugar, as previous studies have indicated that polysaccharides present in liquor, while not themselves contributing color, may influence the transfer of color into the crystal.

The results to date may have implications for the development of color on storage (which appears to be an increasing problem in some areas), and for understanding the transfer of color into white sugar crystals.

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CHARACTERIZATION OF COLORANTS IN SUGARBEET PROCESSING USING GPC WITH ELSD AND DIODE ARRAY DETECTION

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ABSTRACT

First of all, meeting the colour specification for white sugar depends on the colour level in the massecuite from which the sugar is crystallised. The transfer from colorants into the sugar crystals then determines the sugar colour which can eventually be achieved. In this respect the technique of sugar crystallisation and subsequent centrifugation is very important, but the current methodology in modern sugar factories takes rather good care of that. On the other hand, the reason why certain colorants appear to be included in the sugar crystals much easier than other colorants with the same colour intensity at 420 nm, is less well understood. It is suggested that differences in molecular weight of colorants play a particularly decisive role in their transfer to sugar crystals; e.g. the higher the molecular weight, the more difficult it will be for the colorant to diffuse away from the crystallising sugar and so become subject to inclusion in the crystals.

In order to study the molecular weight composition of coloured compounds present in beet sugar manufacture, we adapted formerly presented analytical methods for this purpose. The necessity for optimising the existing methods is that they are usually applied for the analysis of cane colorants, which do have a different nature compared to beet colouring matter. The principle of the molecular weight analysis is a separation of the constituents on an appropriate gel permeation chromatography (GPC) column. Subsequently, the separated components are detected by diode array (DAD) and an evaporative light scattering (ELSD) detector in series. The ELSD gives a good indication of the quantity of the detected components, whereas the DAD enables the characterisation of their spectral nature.

Some examples will be presented on the application of the developed GPC method for the characterisation of beet sugar colorants. Also pre-treatment of samples, such as alcohol precipitation and adsorption to an anion-exchange resin, has been used in order to get a better view of the properties of sugar beet colorants.

INTRODUCTION

Over the years, many research studies have been devoted to the phenomenon of colour in sugar manufacture. Much is already known about the nature of cane and beet sugar colorants. The factors affecting white (beet) sugar colour was excellently reviewed by Shore, *et al.*, (1984). By GPC they demonstrated the presence in juices and beet sugars of low (<100), medium (100-1000) and “high” (1000-5000) molecular weight materials. Additionally, Godshall, *et al.*, (1991) showed that white beet sugars may also contain a proportion of colorants with a molecular weight above 20,000 daltons. The “high” molecular weight colorant may be incorporated preferentially into the white sugar crystal. A minor part of the extracted beet polysaccharides, which can have even higher molecular weights, ends up in white sugar as well (de Bruijn and van Casteren, 1996). It is known that the indigenous polysaccharide from cane can bind with colorant to make a very high molecular weight complex (i.e. the majority of cane colorant is over 300,000 daltons), which occludes preferentially in the crystal (Godshall and Baunsgaard, 2000). Also in beet sugar manufacture, the extracted polysaccharides might become associated with colorant, thus increasing their molecular weight and the inclination to transfer into sugar crystals.

Due to the much higher molecular weight of cane colorants, their transfer (occlusion) into sugar crystals is much more severe than for beet colorants: The occlusion index for cane colour ranges from 10 to 20%, whereas for beet colour usually an occlusion index around 1% is found. In spite of the rather narrow range for both massecuite colour and occlusion index in beet sugar manufacture (typically 2000 ± 300 I.U., respectively 0.9-1.3% for CSM Suiker), the eventually obtained white sugar colour may still vary between 15 and 30 I.U. Taking into account the slow, but gradual colour increase of beet sugar upon storage, the produced white sugar colour should be maintained well below 30 I.U. (e.g. < 25 I.U.) in order to ensure that the specifications for colour can always be complied with. However, it has appeared that we are not always able to keep away from the combination of unfavourable conditions, i.e. both a high massecuite colour and occlusion index, thus resulting in the (temporary) production of off-spec sugar. This in fact has been the main reason to start a study on beet colorant in order to improve our insight into their nature and behaviour. The use of a suitable modern analytical technique is considered to be a requisite for such an in-depth study, which should enable us to distinguish between colorants with probably only small differences in size and chemical structure.

Recently, Bento (2000) reported a study on colour formation in cane sugar refining using GPC with an ELS detector. The described method seemed to be applicable to beet sugar colorants as well, which we therefore initially adopted for our research. However, as will be discussed in this paper, it needed some modification in order to determine beet colorants in a proper way.

MATERIALS AND METHODS

Equipment

GPC was carried out by using either a Superose 12 HR 10/30 or a Superdex 75 HR 10/30 column, Amersham Pharmacia Biotech. The molecular weight separation ranges (for globular proteins) of these columns are respectively, 1000-300,000 and 3000-70,000 daltons. For the Superdex 75 HR 10/30 the fractionation range concerning dextrans is defined as to be 500-30,000 daltons. For detection, both the diode array detector UVD 340S (Dionex) and the PL-ELS 1000 evaporative light scattering detector (Polymer Laboratories), coupled in series, were applied.

Reagents, GPC Conditions and Sample Preparation

According to Bento (2000), initially ammonium acetate (7 mM) in 30 % aqueous acetonitrile solution was used as the mobile phase. At a later stage, as will be indicated below, the mobile phase was changed to 100 mM ammonium acetate. The eluent flow rate was 0.5 ml/min at an ambient column temperature. Samples were injected (200 μ l), after dilution as concentrated as required, or possible (e.g. 20 g of white sugar was dissolved in 50 ml water), and subsequent filtration through a 0.45 μ m membrane.

Different dextrans (Fluka, Sigma) with known average molecular weights (1500, 10,600, 60,000, and 500,000 daltons) were used to calibrate the retention behaviour on the GPC columns.

RESULTS

Method Development

Our major interest concerns the type of beet colorant present in white sugar crystals. We initially applied the GPC method of Bento (2000) for the analysis of white sugar samples with differentiated colours, i.e. ranging from 15 to 60 I.U. A typical example of the obtained chromatogram with diode array detection is shown in Figure 1. The cross-section at 280 nm of the diode array detection together with the ELS detection of the same GPC separation is depicted in Figure 2. It can be observed that after a retention of about 35 min the chromatogram is highly disturbed by the huge sugar peak, eluting at that location.

Dextrans with different (known) molecular weights, sucrose and glucose were separated on the Superose 12 column in order to calibrate the column. Thus, a relation was obtained between the retention time and molecular weight (see Figure 3). Note that so far the GPC separations were carried out on the Superose 12 column using 7 mM ammonium acetate and 30% acetonitrile as mobile phase. The exclusion limit of this column for globular proteins is 300,000 daltons, which agrees with the elution in the void volume of dextran having an average molecular weight of 500,000 daltons. In between the void volume and the sucrose peak the separation of different molecular sizes is as expected. The small molecules, sucrose and glucose, co-eluted.

After a first screening of about 25 white sugars (data not shown), with colours in the range 15 – 60 I.U., we tried to find a relation between the colour peaks observed in the diode array detection and the ICUMSA colour of the sugars. We particularly considered the height of the peaks at different wavelengths. However, we were not able to find the relation we were looking for. Also the (small) ELSD peaks showed no relation with the sugar colour level. There might be several reasons for this: e.g. important information on the colorants is hidden behind the huge sugar peak in the chromatogram, adsorptive retention of colorants by the gel matrix prevents a proper evaluation of the data. We concluded that the current method needed to be further improved.

As mentioned in the introduction, it is known that beet sugar colorants have a relatively low molecular weight, which led us to decide to switch to a GPC column more suitable for the separation of low molecular weight components. For that purpose, we installed the Superdex 75 column, with a fractionation range of 500-30,000 daltons for dextrans, in the GPC system. Using the same mobile phase, i.e. 7 mM NH_4Ac in 30 % acetonitrile, the chromatogram of Figure 4 was obtained for several dextrans with known average molecular weight. As 30,000 daltons is specified for the exclusion limit of this column, the dextran with 60,000 average molecular weight should have appeared in the void volume, just like the 500,000 dalton dextran. Surprisingly, this was not the case.

After consulting Mr. Schols, a specialist in GPC separation of biopolymers at the Wageningen University, the Netherlands, we were advised to increase the ionic strength of the eluent. Also adjusting the pH to below 4 might be helpful in order to prevent interaction of anionic polymers with the gel matrix. Finally, acetonitrile should not necessarily be present in the mobile phase. Based on this advice, we eventually developed a mobile phase which consisted of 100 mM ammonium acetate in water ($\text{pH} \approx 6.8$). We thus obtained the chromatogram in Figure 5 for dextrans, sucrose and glucose, which shows the expected retention behaviour for the different carbohydrates. The 60,000 dalton dextran then correctly appeared in the void volume and even sucrose and glucose were slightly separated from each other. Some preliminary tests, concerning the possible influence of lowering the eluent pH on the separation behaviour of the components under investigation, demonstrated that there is no urgent need to adjust the pH of the eluent. Therefore, we decided to use the 100 mM ammonium acetate solution, just as it is, as eluent for separation of beet colorants on the Superdex 75 column.

Beet sugar colorants

In order to get an idea when beet colorants elute from the GPC column, a rather concentrated beet molasses sample was injected. As the gel filtration medium was packed in a glass column, the course of the colorant during the separation run was visual. (See Figure 6.) The colour band eluted from the column between 30 and 40 min, so around and starting slightly in front of the sucrose peak. This indicated that the molecular weight of these colorants will be < 5000 daltons.

Figure 7 shows the diode array detection of the obtained chromatogram from molasses. Needless to say that beet colorants do not have an absorption maximum at 420 nm, the wavelength which is defined for measuring the sugar colour. In fact, the colour observed at 420 nm in the visible region is just the result of the tail of an absorption peak in the UV region. Combination of the “individual” absorption peaks in the chromatogram of Figure 7 results in the UV/VIS spectrum

beet molasses shown in Figure 8, which clearly confirms this statement. One could imagine that the height of the absorption peaks, the wavelength at maximum absorbency, as well as the slope of their tails influence the final colour measured at 420 nm. It is known that the different colour types (i.e. melanoidins, caramels, hexose alkaline degradation products) do have different absorption maxima and, thus the shape of their respective absorption curves may also differ.

In spite of the improved separation of polymers using the Superdex 75 column, essentially in the lower molecular weight region, there still was some overlap around 35 min between sucrose and colorants. Furthermore, retention of colorants after the sucrose peak was observed. Theoretically this means that they should have a molecular weight between 0 and 342 dalton. There might be beet colorants with such a low molecular weight, but it cannot be excluded that certain colorants do interact somehow with the gel matrix, thus demonstrating an incorrect, artificial low molecular weight.

We tried to get rid of the sucrose by alcohol precipitation, i.e. 80 % alcohol and 0.1 % NaCl, of (colorant) polymers in beet molasses. It turned out that less than 25 % of the colorant could be separated in this way; in other words, the majority of the beet colorants is well soluble in alcohol and therefore will have a relatively low molecular weight, which in fact agrees with earlier findings.

Figure 9 compares the chromatogram of the alcohol precipitate (after it was dissolved in water) and that of the original molasses using ELS detection. The peak around 39 min retention time may be due to salt components, the large peak around 36 min originates from sucrose. In the chromatogram of the molasses sample, a front peak could be observed as a shoulder on the sucrose peak. In the alcohol precipitate, this peak, with an estimated molecular weight of about 1500 daltons, clearly shows up. Additionally, in the void volume a high molecular weight colorant > 30,000 daltons also appears to be precipitated by using alcohol.

Another possible approach to separate the colorants from sucrose was a treatment with ion exchange resins. In a trial we added a molasses sample on top of a small glass column filled with strong cation exchange resin, which was connected in series with a column filled with strong anion exchange resin. It could be seen that the colour went through the cation exchange resin, but was caught by the anion exchange resin. In order to desorb the anionic colorants, after washing with water, the column was flushed with 5 (m/m) ammonium carbamate, which can be removed afterwards from the effluent by evaporating the ammonia and carbon dioxide. However, the light yellow effluent appeared to contain less than 10 % of the original colour, which means that almost all the colorants were tightly bound to the anion exchange resin. A subsequent flushing with 10 % methanol was not able to remove any colorant either, thus emphasising the anionic character of the colorant rather than a hydrophobic interaction to the backbone of the exchange resin.

By using for instance sodium hydroxide or sodium chloride it should be possible to desorb the colorants from the resin; however, then the collected colorants in the effluent will also contain inorganic salts. As the aim of a sample preparation prior to GPC analysis should be the removal of sucrose from the colorants, replacement of sucrose by another contaminant (like salts), which will also interfere in the GPC analysis, is undesirable and makes the sample preparation useless.

As an example of a colorant that will be present among beet sugar colorants, a preparation of the so-called hexose alkaline degradation products (HADP) was analysed on the GPC system. The chromatogram in Figure 10 demonstrates that the molecular size of this type of colorant was rather small too, i.e. < 5000 daltons.

As a consequence of the necessity for further method development with respect to the specific analysis of beet colorants, we have not yet been able to test the actual developed method on the Superdex 75 column, using just 100 mM NH₄Ac as eluent, for the purpose of determination of colorants in white sugar samples. We only have yet been able to analyse a low and a high colour white sugar sample as examples (Figure 11). Unexpectedly, the low colour sugar hardly showed any peaks by the diode array detection, particularly when it was compared to the earlier chromatogram of the same sugar on the Superose 12 column (Figure 1). The sugar having a colour of 100 I.U. again showed a peak in the 1000-5000 dalton region, whereas a very weak absorption could be observed in the void volume, thus indicating the presence of a tiny amount of high molecular weight colorant.

DISCUSSION AND CONCLUSIONS

The use of modern GPC separation of colorants, assisted by both diode array and evaporative light scattering detection, looks very promising for a further elucidation of the nature of (beet) colorants. It has turned out that the already applied GPC method for cane colorants by Bento (2000) requires certain modifications, in order to make it of use for a study on beet colorants. Although adjustments of the original method, as described in this paper, have improved its applicability in the right direction, we are still not convinced that the actual developed method will be able to give the proper answers to our rather detailed questions regarding the preferential transfer of certain beet colorants into sugar crystals.

Nevertheless, we already have made substantial progress and still see possibilities for achieving further improvements. In the near future we will investigate the potential of ultra-filtration as a matter of sample preparation, which may be very helpful to remove the surplus of sucrose present in samples from sugar manufacture and, additionally, is considered to be a way of pre-concentrating the colorant polymers. Concerning the GPC separation, it would be interesting to further study the effect of the eluent pH on both the separation behaviour and detector response of colorants.

Last but not least, the work carried out so far has confirmed earlier findings concerning beet colorants, particularly with respect to their relatively low molecular weight compared to that of cane colorants. Furthermore, the anionic nature of the beet colorants has to be emphasised.

ACKNOWLEDGEMENT

The authors wish to thank Mr. Luis Bento for performing some preliminary tests and his kind gift of self-prepared HADP.

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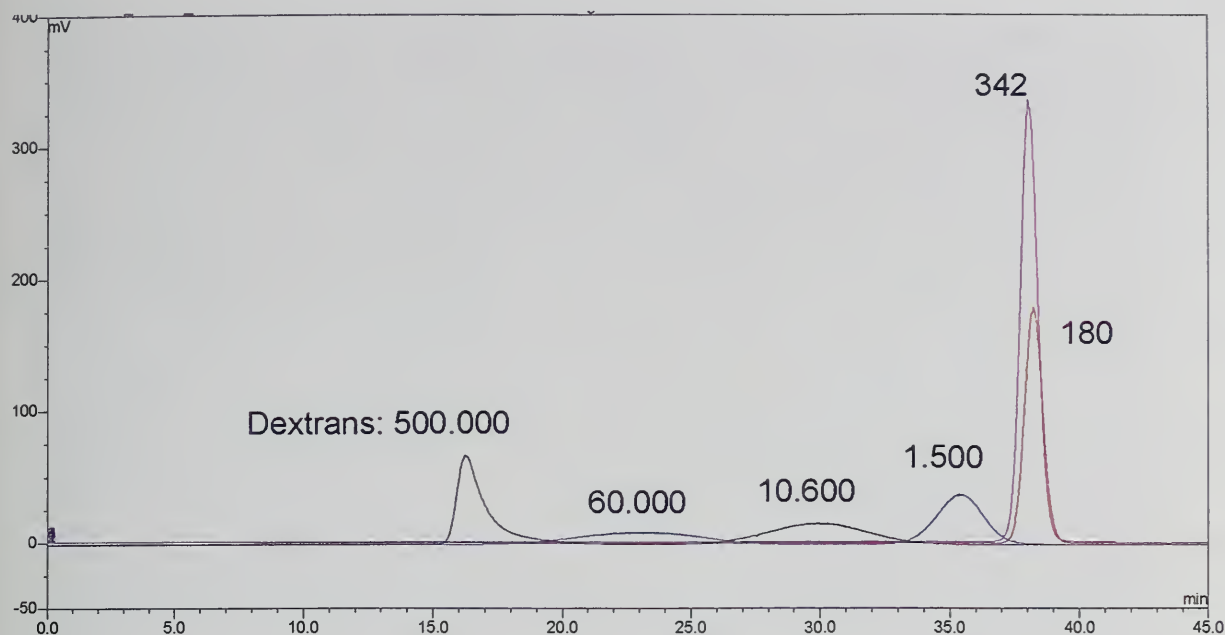


Figure 3. Calibration of Superose 12, eluent 7 mM NH_4Ac in 30 % acetonitrile at 0.5 ml/min, based on the separation of different dextrans, sucrose and glucose

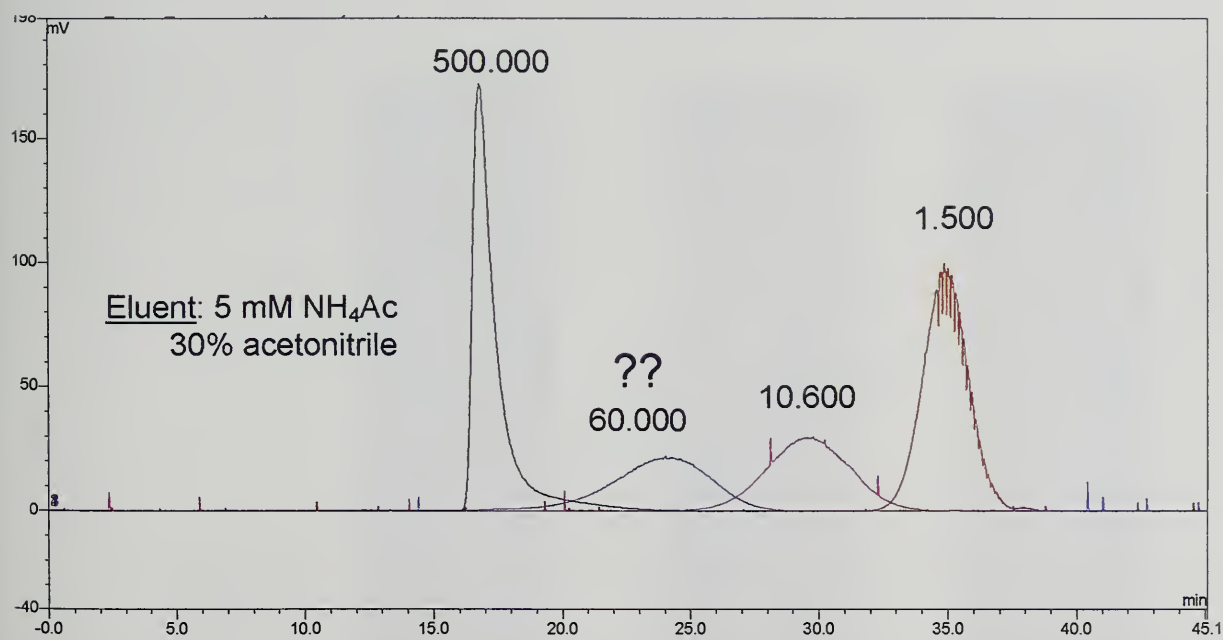


Figure 4. Calibration of Superdex 75, eluent 5 mM NH_4Ac in 30 % acetonitrile at 0.5 ml/min, based on the separation of different dextrans

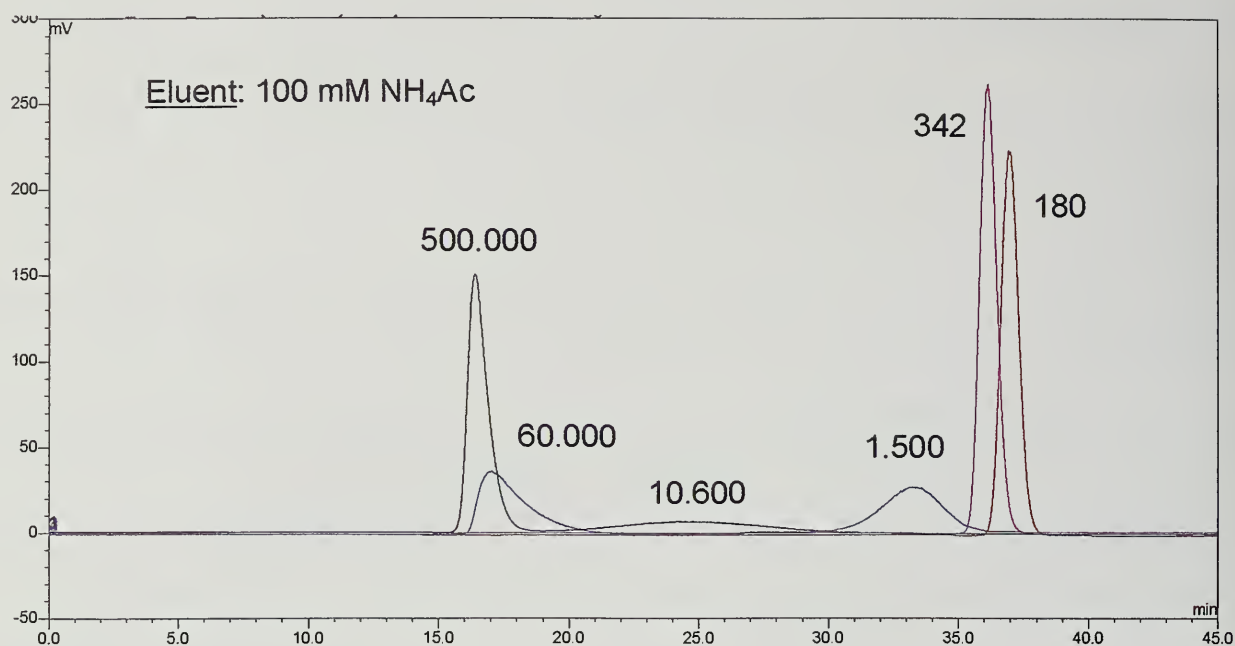


Figure 5. Calibration of Superdex 75, eluent 100 mM NH₄Ac at 0.5 ml/min, based on the separation of different dextrans, sucrose and glucose

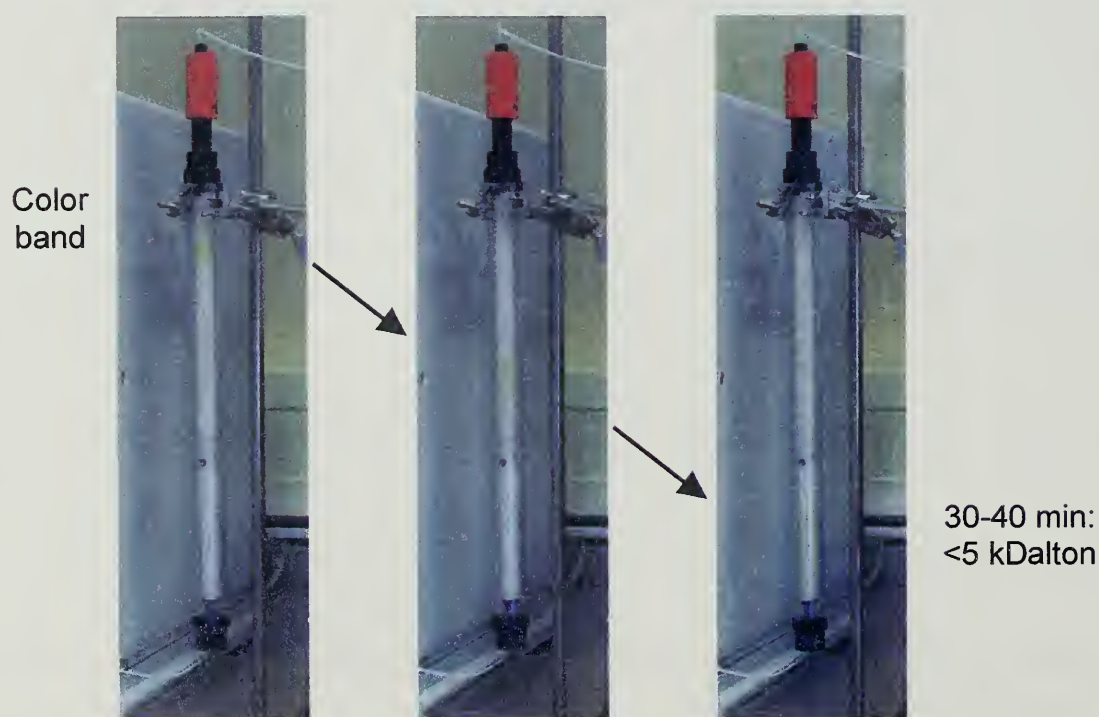


Figure 6. Visualised separation of colorants in beet molasses on Superdex 75 (eluent 100 mM NH₄Ac at 0.5 ml/min).

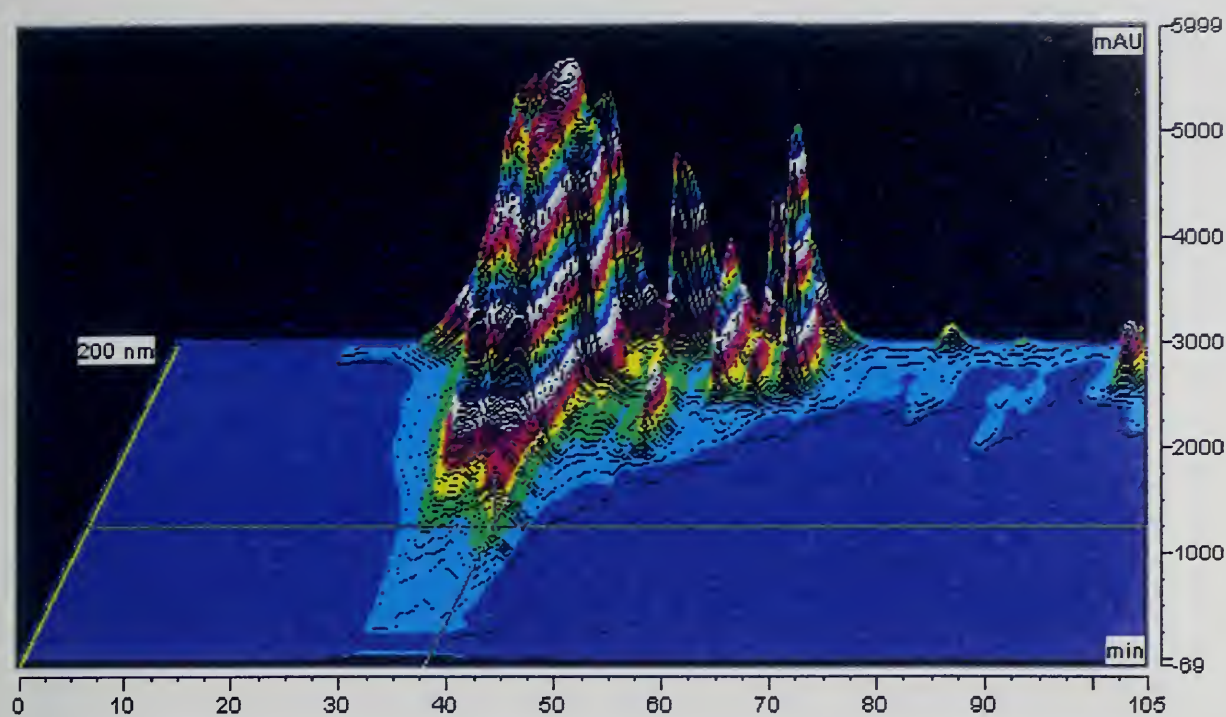


Figure 7. GPC-DAD of molasses on Superdex 75 (eluent 100 mM NH_4Ac at 0.5 ml/min).
The black horizontal line in front of the chromatogram indicates 420 nm on the Y-axis.

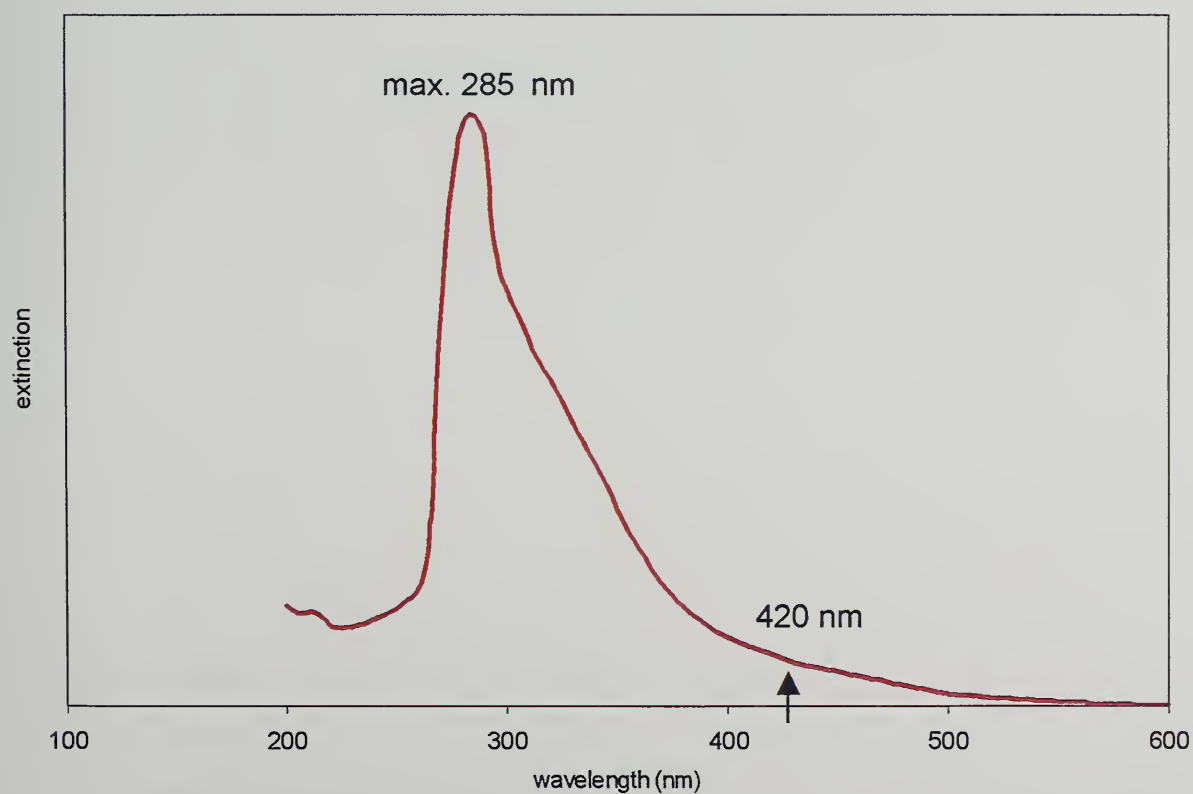


Figure 8. UV/VIS spectrum of beet molasses.

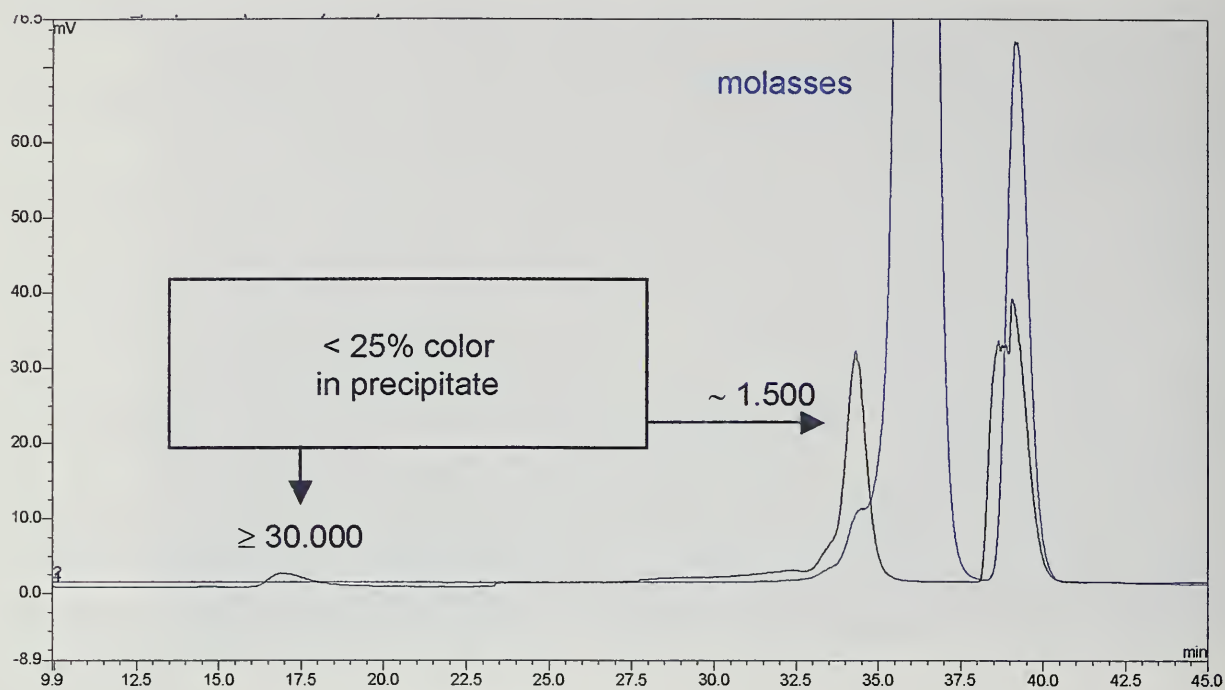


Figure 9. GPC-ELSD of molasses (blue line) and alcohol precipitate of molasses (black line) on Superdex 75 (eluent 100 mM NH_4Ac at 0.5 ml/min).

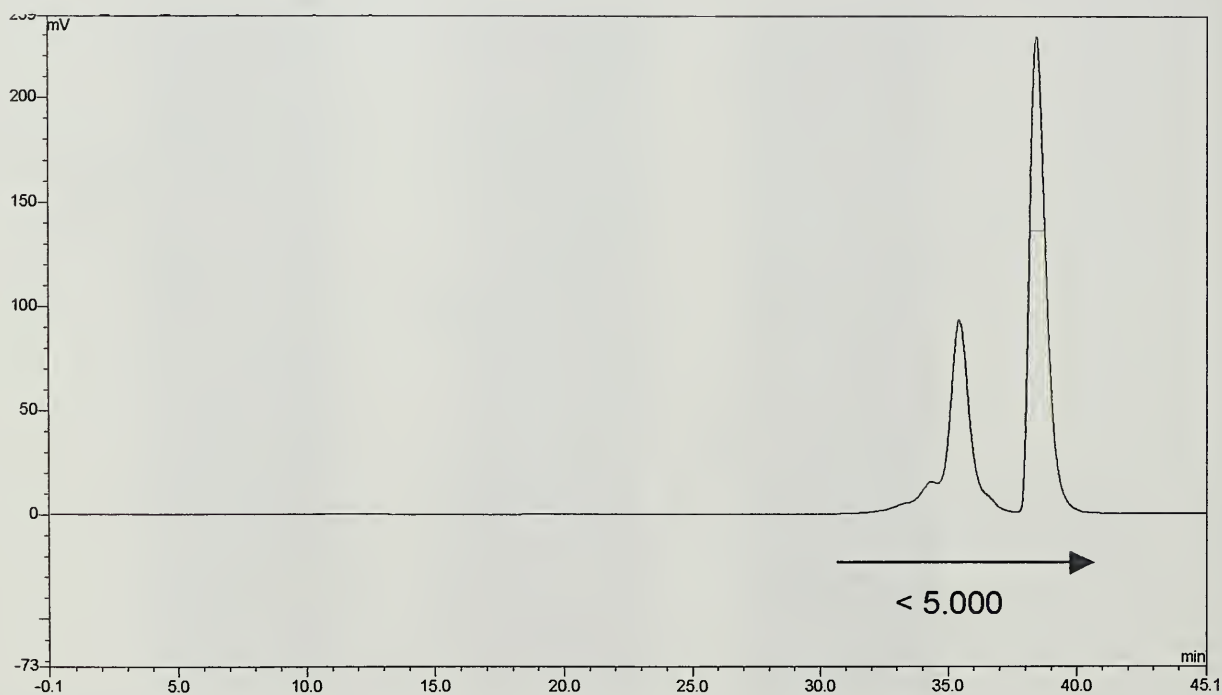


Figure 10. GPC-ELSD of HADP on Superdex 75 (eluent 100 mM NH_4Ac at 0.5 ml/min).

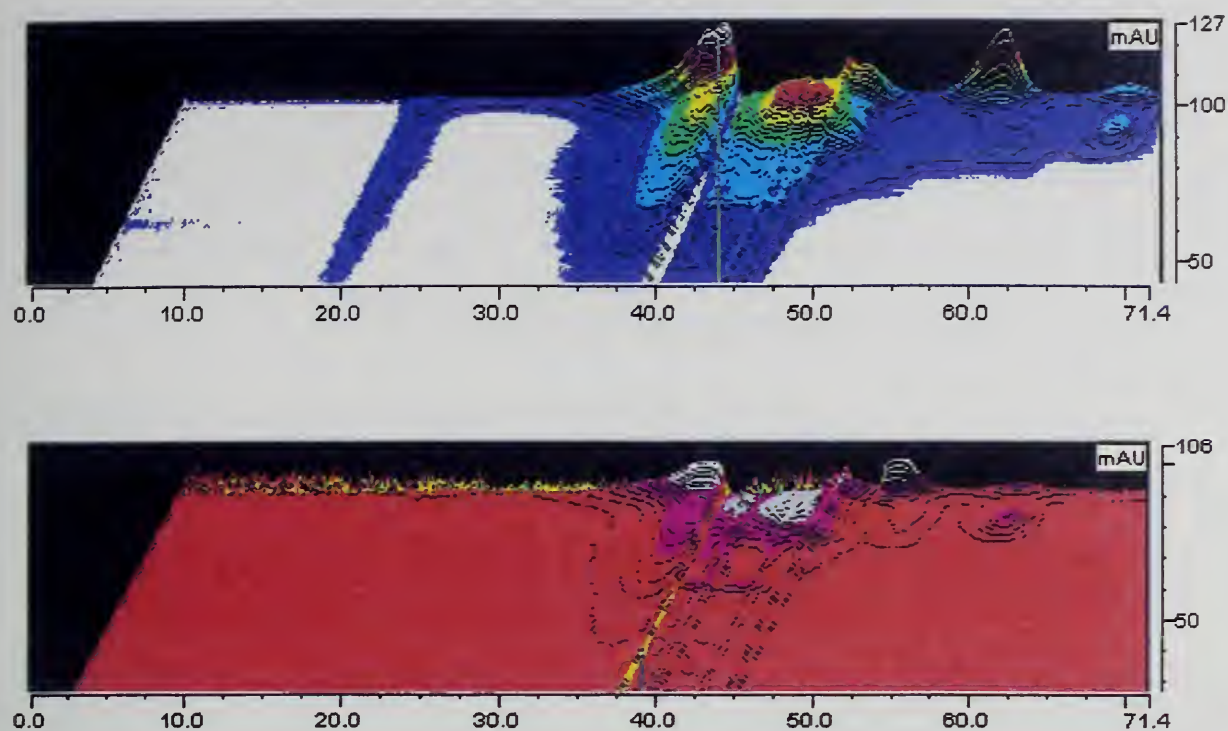


Figure 11. GPC-DAD of white sugar samples with high (100 I.U.; upper chromatogram), respectively low colour (20 I.U.; lower chromatogram) on Superdex 75 (eluent 100 mM NH_4Ac at 0.5 ml/min).

THE CHARACTERIZATION OF CANE SUGAR COLORANT

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ABSTRACT

Sugar juices in the production of cane sugar contain colored material of which little is known. This study aims to provide information about the products of various color formation mechanisms. Color has been formed in the laboratory by alkaline degradation, caramelization, the Maillard reactions, and exposure to iron in an attempt to replicate color formation in the factory. Samples were analyzed with HPLC gel permeation chromatography (GPC), using a differential refractometer to detect concentration in series with an absorbance detector set to 420nm to detect color. GPC produces concentration and color profiles as a function of molecular weight, which enables the effects of these formation mechanisms to be compared. Molecular weight ranges were chosen for the characteristic products of each mechanism. Fractions corresponding to these molecular weight ranges were collected from repeated GPC runs. Fourier-Transform Infrared (FTIR) spectrometry was conducted as a preliminary functional analysis.

INTRODUCTION

Research into the complex organic nature of cane sugar colorants has been a major area of interest in the sugar industry since its beginning. Understanding more about the character of color allows for fine-tuning existing separation processes and for the design of new and better techniques for its removal.

Colorants are often named from their mechanisms of formation. Caramelization and alkaline degradation are similar thermal mechanisms except that alkaline degradation occurs at high pH and forms much darker colorant (Godshall, 2000). The Maillard reactions occur throughout the factory and have many complex pathways (Van der Poel, *et al*, 1998). Reactions proceed under almost all conditions as reducing sugars and amines or amino acids are always present except in the purest of solutions. Iron also plays an important role, particularly in plant-derived colorants (Godshall, 1996). Many polyphenolic compounds are able to produce highly colored iron complexes.

The purpose of this paper is to characterize the products of these color formation reactions as they may occur in the factory. Gel Permeation Chromatography (GPC) is a reliable method to investigate molecular weight effects of each mechanism (Saska and Oubrahim, 1987). Fourier-Transform Infrared (FTIR) spectrometry has been performed on the caramelization and alkaline degradation GPC fractions to yield information on the changes in functionality of particular molecular weight ranges. It must be noted from a purely chemistry perspective that an increase in color should occur as double bonds are formed.

MATERIALS & METHODS

Materials: Evaporator syrup was obtained from the Cinclaire mill for caramelization and alkaline degradation tests. Molasses was obtained from stock at the Audubon Sugar Institute for investigation of the Maillard reactions. Cane juice was produced by disintegrating cane with water in a stainless steel environment using a Jeffco disintegrator.

Caramelization and Alkaline Degradation: Syrup was boiled under constant reflux in an atmospheric laboratory still for 30 minutes. In the case of alkaline degradation, the syrup pH was increased with sodium hydroxide to pH 8.8.

Maillard Reactions: Conditions favoring the Maillard reactions (Newell, 1979) were used: high temperature and brix but low purity. Molasses was maintained at 75° C in a constant temperature bath for 24 hours.

The Effect of Iron on Cane Juice: Cane juice was heated at 50° C in a water bath for one hour. The effect of iron on cane juice was investigated by placing rusty and chemically cleaned coiled wire of equal lengths into the heating tubes. Non-enzymatic effects were investigated by autoclaving the juice (at 110° C for 10 minutes) prior to exposure to iron and also by the addition of one part mercuric chloride to 5,000 parts of juice to denature any enzymes (Meade, 1963). For each treatment, a control experiment was performed to check the effects without any iron in contact with the juice.

GPC Sample Preparation: Dilution to 7 brix
Filtration with a 5.0µm syringe filter

Godshall, *et al.*, (1988) showed that a 0.45µm membrane removes very high molecular weight material. This was confirmed by GPC analysis. A 5.0µm membrane filter was found to be sufficient to remove insoluble material but not remove any dissolved high molecular weight material.

Gel Permeation Chromatography: Reaction products were analyzed with gel permeation chromatography (GPC). A Bio-Rad AS-100 HRLC autosampler was used to inject 100µl of sample into a mobile phase of 0.1M sodium nitrate (NaNO₃), pumped isocratically at 0.5ml/min by a Waters 515 HPLC pump. Separation was achieved using two Waters Ultrahydrogel HPLC columns (Linear and 120) in series to give a molecular weight (MW) range of 6,000,000 to 100.

A Dionex AD20 absorbance detector set to 420nm was used to determine color and a SpectraSYSTEM RI-150 differential refractometer to measure color. Fractionation was performed with a Gilson 201 fraction collector. Each unit was computer controlled using the Dionex Peaknet system (Version 4). Dextran standards, sucrose and water were used to generate a molecular weight calibration curve (Figure 1).

FTIR Spectrometry: The GPC mobile phase was changed to potassium chloride when fraction collecting, as it is invisible to infrared light. Composites of repeated fractioning runs were evaporated to dryness in a vacuum oven. A mortar and pestle were used to grind and mix 5mg of sample with 120mg of potassium bromide (KBr) powder. The mixture was then compressed into a clear crystal in a pellet press. Spectra were subsequently produced with a Nicolett FTIR spectrometer.

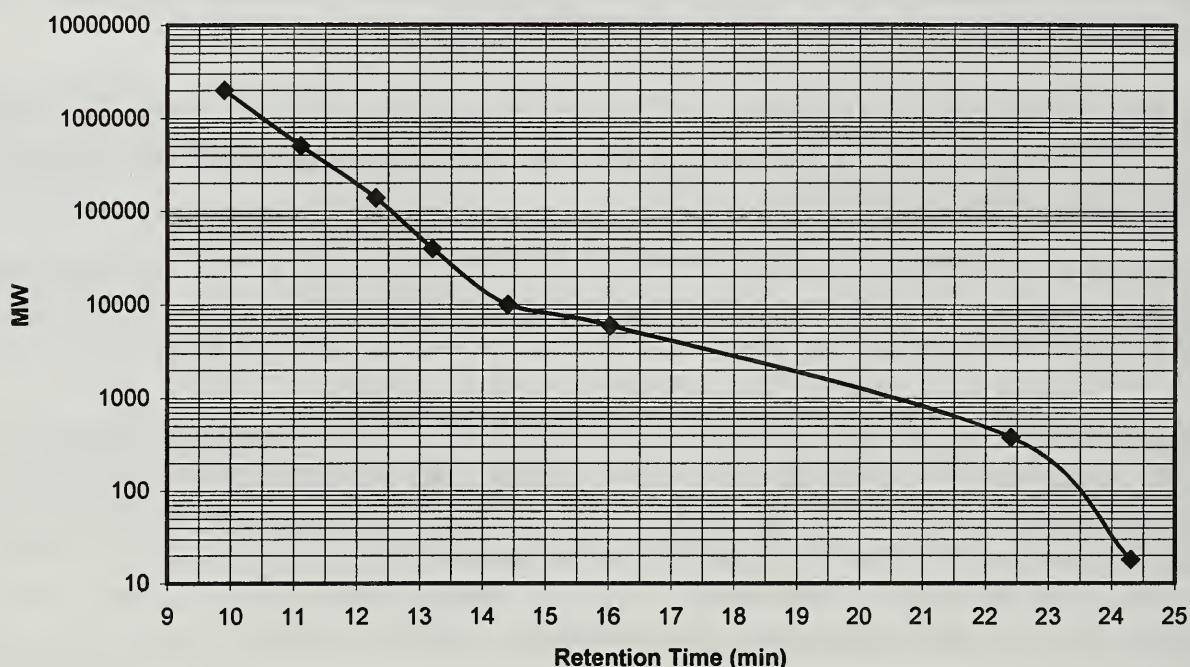


Figure 1. GPC Molecular Weight Calibration

Other Analytical Equipment:

Spectronic Genesys 2 Spectrophotometer
 Bellingham and Stanley Ltd. RFM90 Refractometer
 Orion 410A pH meter

Chromatogram Analysis: GPC is a separation process based on molecular size. A small sample is injected into a stream of a buffer solution that flows into a precisely controlled pore size gel column. The gel pores are arranged in such a size distribution that some small material is able to diffuse into the pores whereas larger molecules are excluded. The column may be calibrated by injecting standards of precise molecular weight into the column. If the samples to be analyzed are of the same molecular size and shape as the standards, their weights may be read off the calibration curve. The buffer solution masks the gel from any ionic behavior of the sample, as no interaction is wanted between the analyte and the stationary phase.

Interpretation of a chromatogram was performed by numerically integrating the peaks into areas. Standardization was performed by developing a relationship between peak area and concentration for the refractive index detector and peak absorbance from the absorbance detector (420nm). After picking the peaks, numerical integration was performed. Choosing the appropriate peak split times was often complicated. Normally a peak split point was chosen by a zero derivative but in the high molecular weight ranges, this was difficult to pick out. Peak split points were chosen by closely looking at the refractive index chromatogram. Figure 2 shows a characteristic chromatogram and the peak split points chosen. It must be noted that as long as the peak split-times remain constant the peak areas may be compared with those of other samples.

A MATLAB (Version 6) computer program was created to integrate the chromatograms as the Peaknet software did provide enough flexibility. The basic algorithm used is listed on the next page.

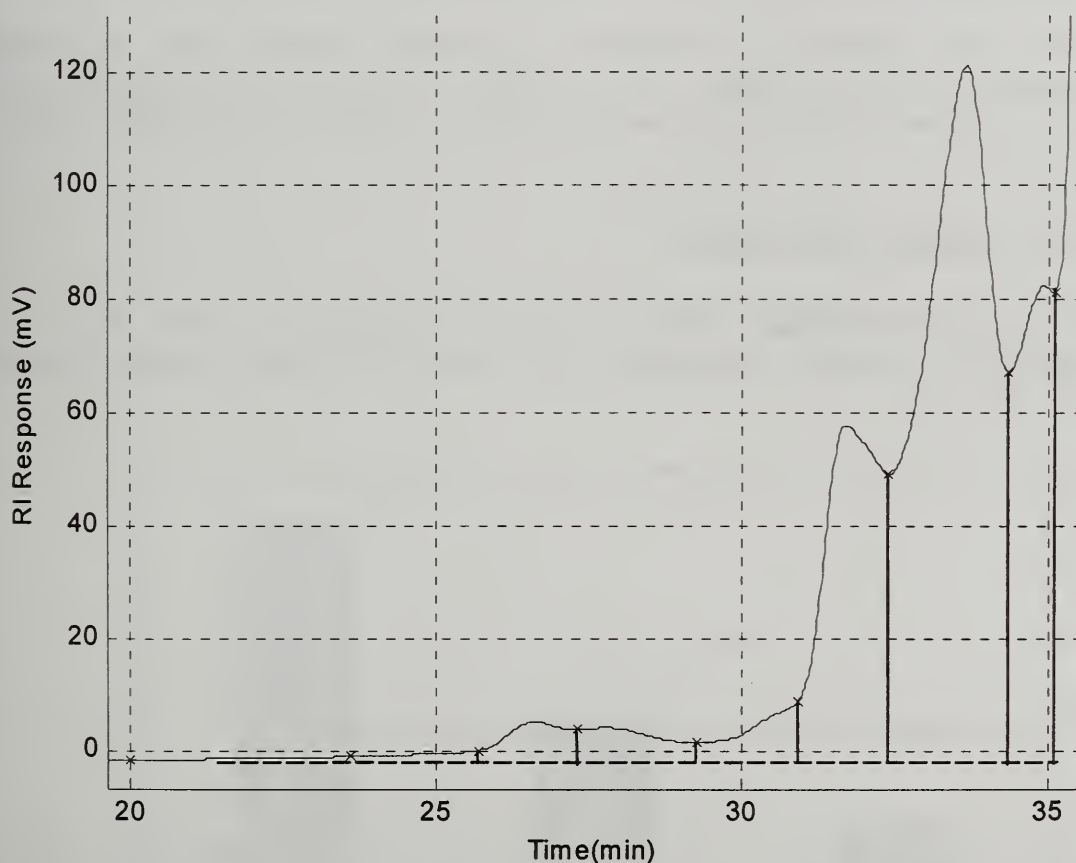


Figure 2. A typical GPC Refractive Index Chromatogram

Integration Algorithm:

1. Determine location of data-files (saved as ASCII text)
2. Load and display data
3. Allow the user to indicate the region of the chromatogram to be used for the baseline
4. Calculate the average response over this region
5. Display the peak-split points from the previous chromatogram
6. Allow the user to zoom in on the region of interest
7. The mouse pointer is used to adjust these points
8. The area between baseline and response curve is calculated by numerical integration. The trapezoidal method is sufficient as many data-points were collected)
9. The results are stored and the chromatogram printed
10. Index to the next sample → Back to 2

RESULTS AND DISCUSSION

The results of the color formation experiments are presented starting from the simplest measurement technique, ICUMSA Color. This is followed by the more informative GPC analyses. For three fractions of initial syrup, caramelization and alkaline degradation FTIR data are presented.

Caramelization and Alkaline Degradation

Simple ICUMSA Color measurement shows a threefold increase in color for alkaline degradation, considerably more than for caramelization owing to the harsh reaction conditions (See Figure 3).

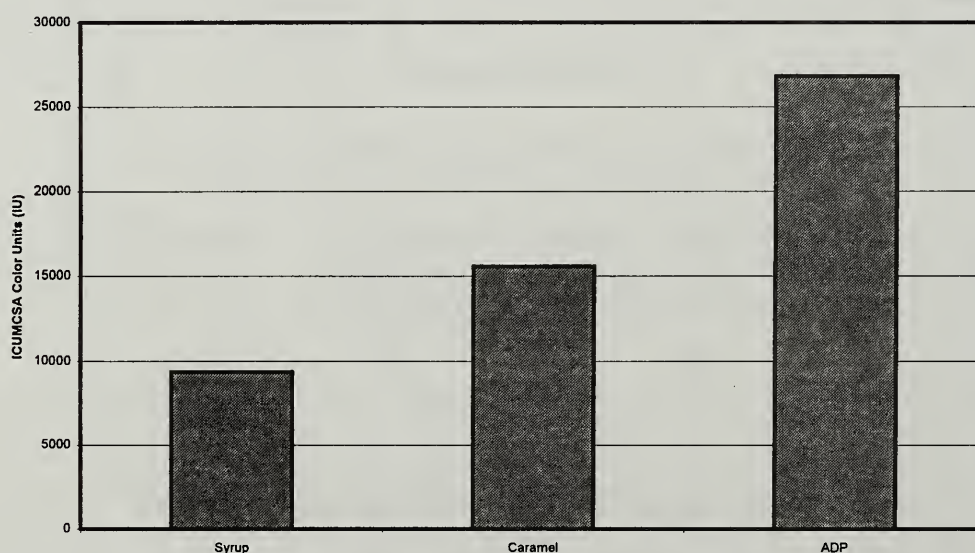


Figure 3. ICUMSA Color of Caramelization and Alkaline Degradation

GPC is a more insightful analysis into the formation of sugar colorants. The resulting refractive index (RI) chromatograms are overlaid in Figure 4(a). Figure 4(b) shows the region of interest, since sucrose overloads the detector the peak may be ignored.

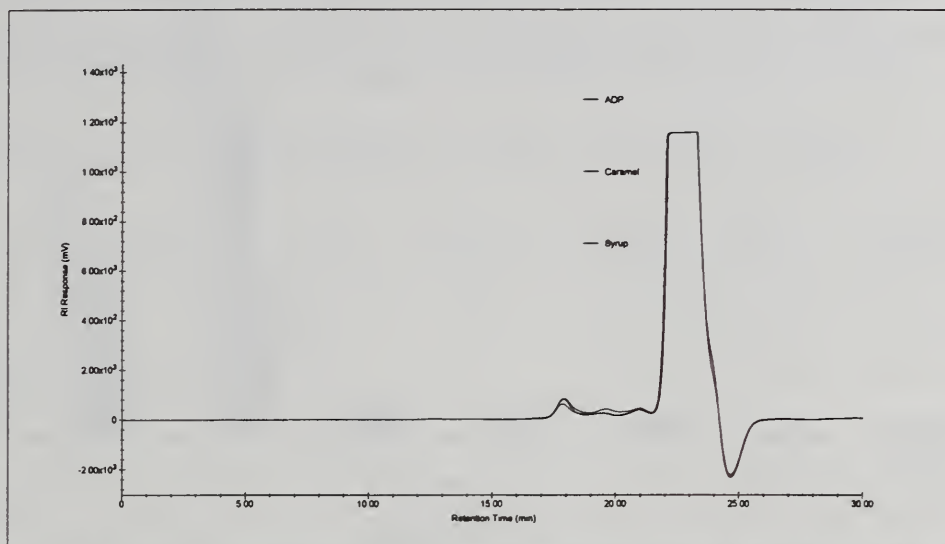


Figure 4(a). RI GPC chromatograms for Caramelization and Alkaline Degradation

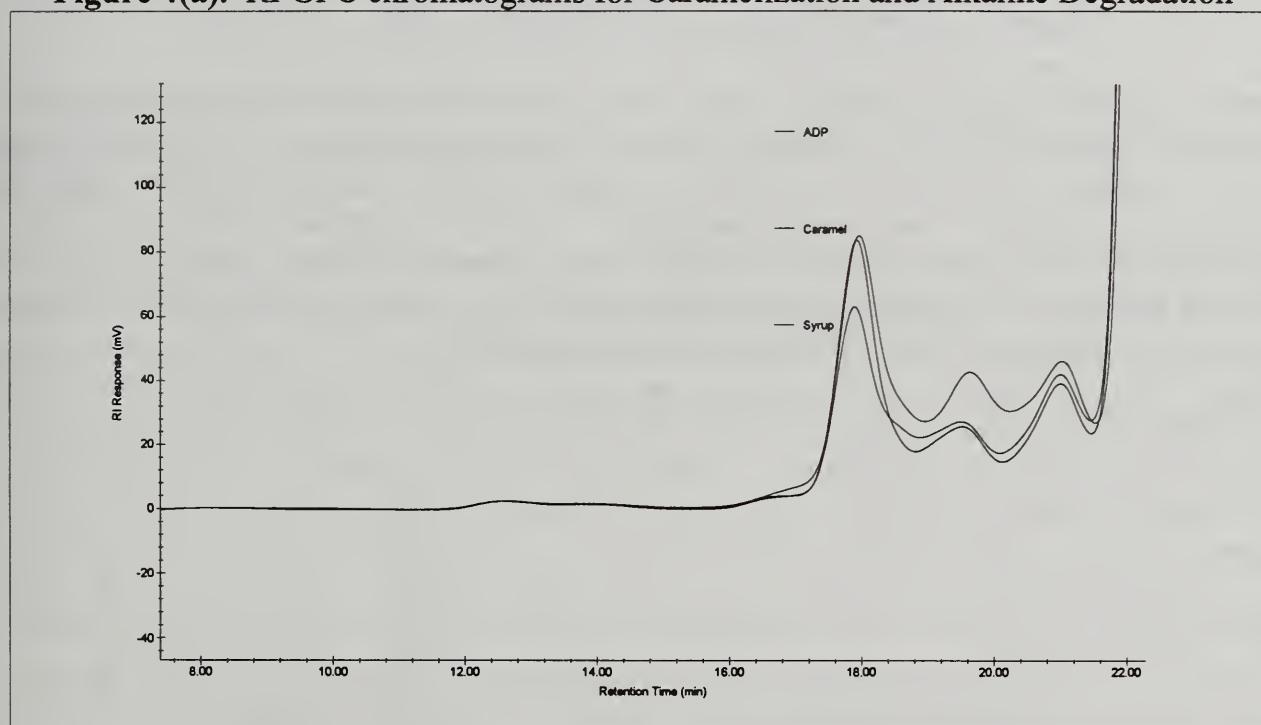


Figure 4(b). Region of Interest in GPC Chromatograms

The peaks identified from the chromatograms were compared. By comparing the molecular weight ranges identified with the initial syrup, the concentration effects of caramel and alkaline degradation product (ADP) mechanisms as a function of molecular weight may be determined. The integrated results are displayed as a bar chart in Figure 5.

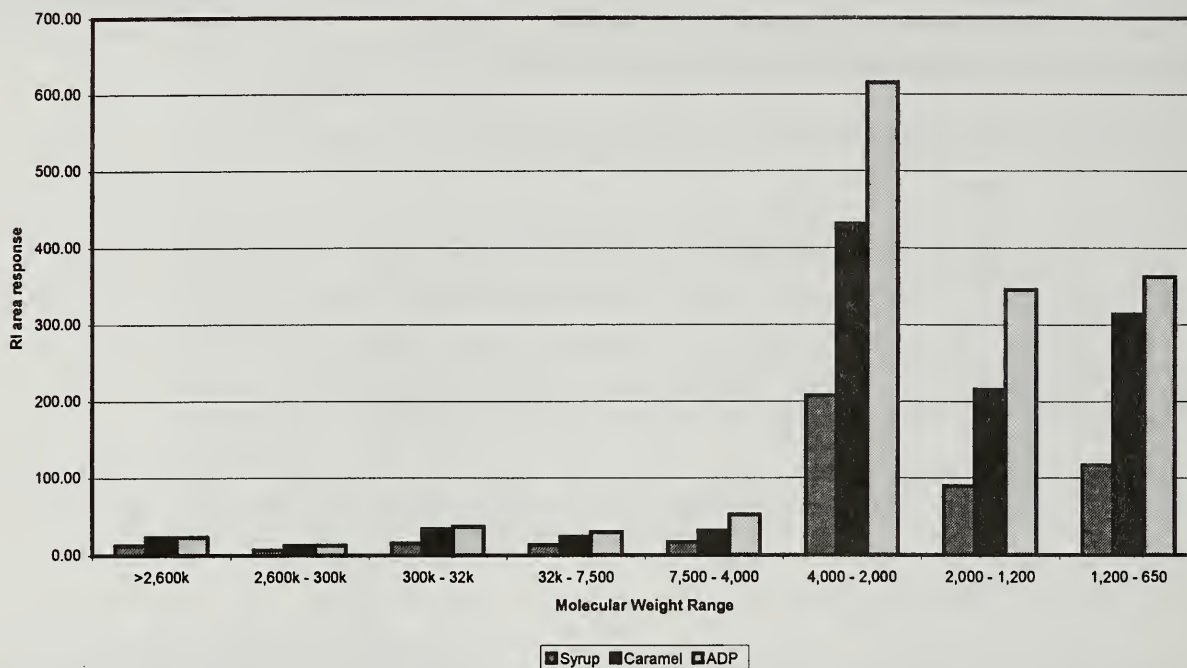


Figure 5. Caramelization and Alkaline Degradation – RI Areas

Increases in concentration are noticeable in all ranges showing that material in the range of sugar molecular weights (<650MW) is being polymerized into larger molecules. This explains why such large increases are noticed in the lower ranges. In all the ranges, alkaline degradation produces more material. The color chromatograms produce a similar result (see Figure 6), except that ADP's show more highly colored than the caramel products. Figure 6 shows that ADP's and caramels are produced from material of molecular weight less than 650 as increases are viewed in all ranges. Clearly, sugars are being polymerized.

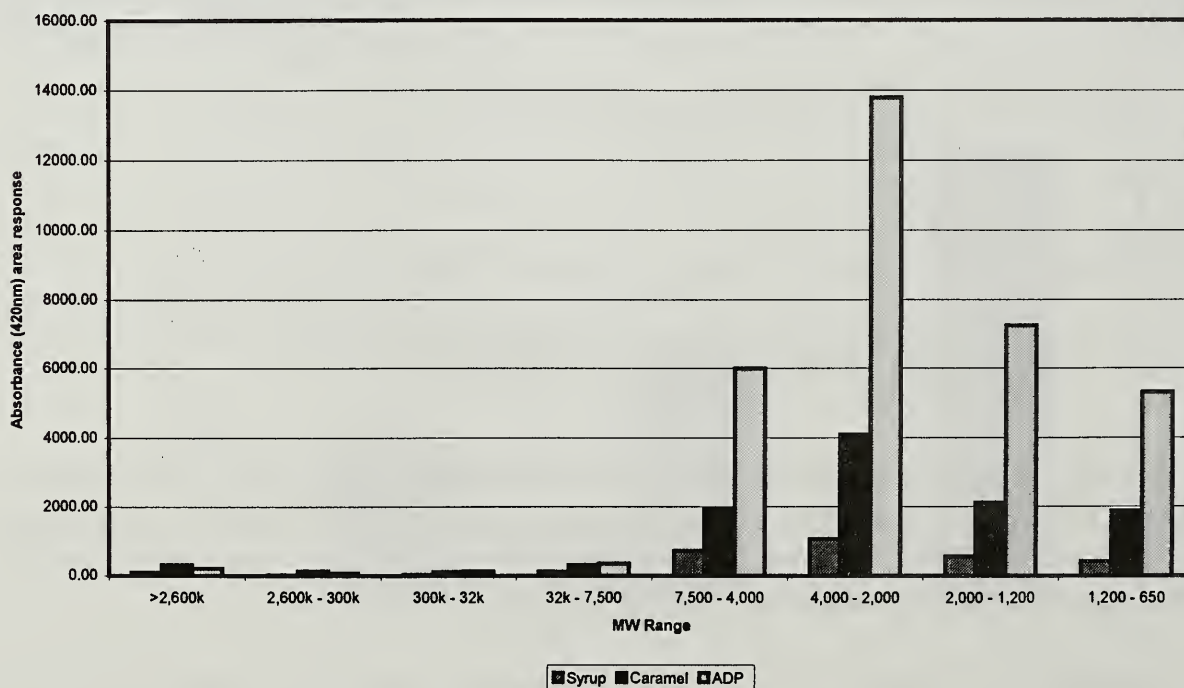


Figure 6. Caramelization and Alkaline Degradation - Absorbance at 420nm Response

HPLC analysis of the samples was performed, analyzing the organic acid concentrations. The difference between caramelization and alkaline degradation is strikingly different. Alkaline degradation causes the formation of organic acids. In the thirty-minute period every acid except for aconitic acid, approximately doubled its concentration. In contrast, during the caramelization reactions, the concentrations of all the organic acids reduce markedly.

Table 1. Organic acid concentrations (ppm) in caramel and ADP formation

Sample	Acetic	Aconitic	Citric	Formic	Lactic	Malic	Oxalic	Propionic
Syrup	1040	2999	310	220	1418	413	33	43
Caramel	687	1141	186	153	937	234	19	n/d
ADP	2058	3243	365	437	2392	492	108	82

n/d – non-detected

In FTIR spectrometry, nitrates create a broad intense peak over the wavenumber range of interest. The GPC mobile phase was changed to 0.1M potassium chloride (KCl), as this salt is invisible to infrared light. KCl is not an ideal mobile phase as the retention time of the material is affected by the amount injected. Repeatability of the same sample was, however, not a problem so KCl mobile phase peaks were matched to the NaNO_3 peaks (there was a 30 second shift in retention times). Solid-phase FTIR is a lengthy analytical technique as forming the KBr plates is difficult. In this preliminary analysis, only fractions 6, 7 and 8 were analyzed (4,000-2,000 MW, 2,000-1,200 MW & 1,200-650 MW).

FTIR is usually used with pure components. The exact configuration of the intensity and wavenumber (analogous to frequency) of the peaks may be matched with a spectral library for compound identification. To test the analysis, the spectrum of a sucrose sample was generated (Figure A.1). This closely matched the sucrose spectrum in the spectral library. Naturally produced biopolymers have a large polydispersity as the reactions do not take place under perfectly controlled conditions. This means that it is impossible to match the IR spectra generated from the GPC fractions with specific compounds. A compound such as dextran with a 6,000 MW average (Figure A.2) is not recognized by the spectral library as it contains many different length polymers. Despite this, FTIR does yield a lot of information about the functionality of the material. Table 2 lists the wavenumbers of the functional groups identified. When using FTIR it is important to take the background spectrum as peaks occurring in the samples may just be the background. A KBr plate was analyzed to achieve this (Figure A.3). At 667cm^{-1} a large spike appears and so in the analysis of the samples this peak must be ignored. In addition at $2,361\text{cm}^{-1}$, noise is obtained that must again be ignored. In the region around $1,600\text{cm}^{-1}$ noise is also prevalent, but as long as the sample peaks in this range are large enough it may be ignored.

Table 2. Characteristic infrared absorption frequencies (Morrison & Boyd, 1992)

Bond	Compound Type	Frequency Range, cm^{-1}
C-H	Alkanes	1350-1470
C=H	Alkenes	1640-1680(v)
C-O	Alcohols, ethers, carboxylic acids, esters	1080-1300
C=O	Aldehydes, ketones, carboxylic acids, esters	1690-1760
O-H	Hydrogen-bonded alcohols, phenols	3200-3600(b)

(b) - Broad peak

(v) - Variable: conjugated double bonds appear at slightly lower wavenumbers

Each fraction will be considered independently. Figure A.4 shows the spectra obtained for fraction 8 (1,200-650MW). As mentioned earlier, formation of double bonds will cause increased color in the samples. It is clear from the spectra that caramelization is increasing the number of alkene (C=C) bonds. Alkaline degradation shows a more pronounced effect particularly for carbonyl (C=O) bonds. It is interesting to note that no aromatic material is being observed in its characteristic range as indicated in the figure.

Figure A.5 shows the spectra of fraction 7, the 2,000-1,200 MW range. Caramelization shows a large increase in all ranges (O-H, C=O, C-C and C-O). At first glance, the ADP curve appears to contain a lot less double bonds than the caramels. Figure 6 plainly shows, for this range, that the color increase is greater for alkaline degradation than for caramelization. The conflict is resolved by comparing the ratio of double bonds (C=O and C=C) to single bonds (C-C), that is color inducing bonds to non-color inducing bonds. ADP's clearly have a higher ratio of double bonds to single bonds. This explains why ADP's are darker than caramelization products.

For higher molecular weights, the concentration in the GPC eluent is very low and so many samples are composited to obtain enough material to analyze with FTIR. The KCl GPC buffer salt dilutes the amount of solid material obtained. Two options are possible to increase the concentration: dialyze out the salt or use a higher ratio of sample to KBr when making up the plates. Owing to time restrictions, this was not completed for this paper. This problem is seen clearly in Figure A.6 for fraction 6 (4,000 to 2,000 MW) as the peaks are very small, any higher fractions give almost no response at all. It is pointless to draw conclusions from this range as the FTIR spectrometer is approaching its detection limit.

Maillard Reactions

The Maillard reactions were analyzed as in the previous experiment except FTIR was not performed. The present FTIR analysis is lengthy and will be shortened when the Department of Chemistry at Louisiana State University has its liquid cell capable FTIR repaired. This will remove the drying and plating steps allowing for rapid analysis.

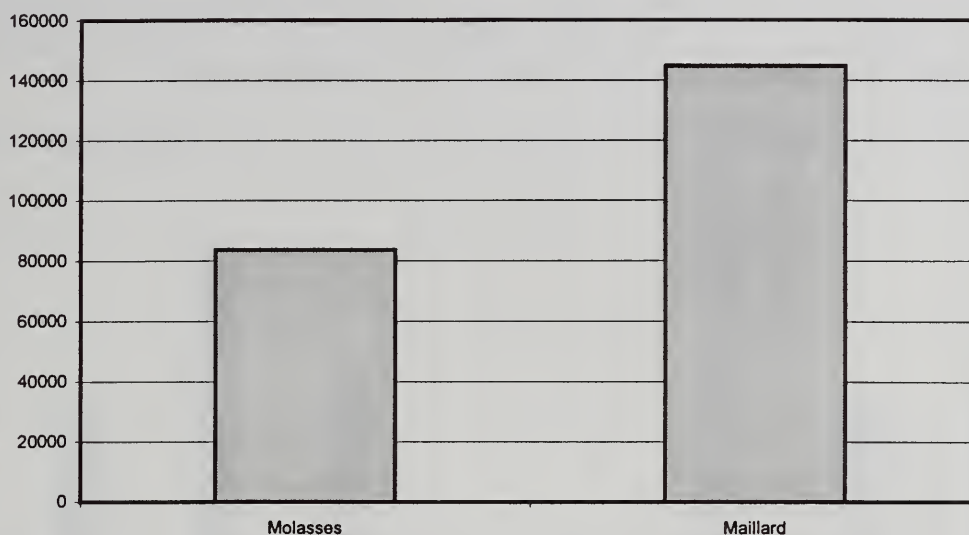


Figure 7. Increase in ICUMSA Color from the Maillard Reactions

It is interesting to note that the same molecular weight ranges were obtained for the Maillard reactions as for ADP and caramelization, except that the highest range had to be extended. Substantial increases in concentration are seen in all ranges (Figure 8).

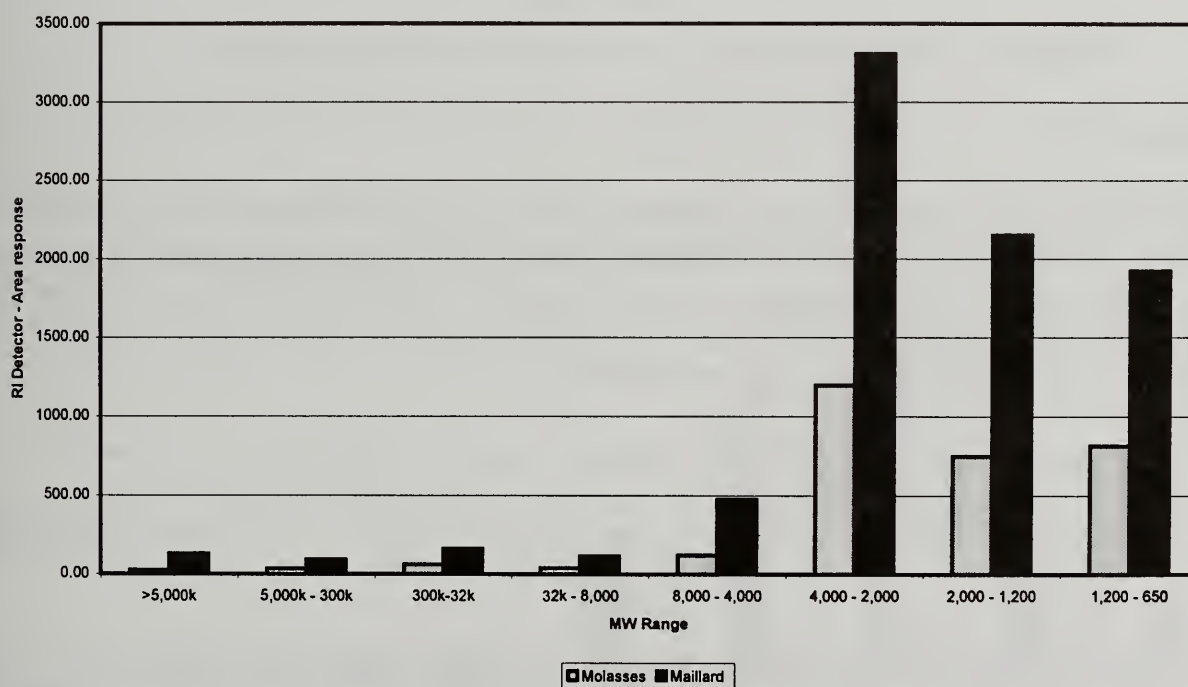


Figure 8. Maillard Reactions – RI Areas

Figure 9 shows how the high molecular weight ranges contain insignificant amounts of color in this reaction compared to the ranges, 32k MW and below. It is interesting to compare the ICUMSA color data with GPC data. A greater increase in the absorbances (Figure 9) is seen compared to the ICUMSA color results (Figure 7). This is a result is caused by ICUMSA color

compared to the ICUMSA color results (Figure 7). This is a result is caused by ICUMSA color being an intensity parameter: the color per unit dissolved solid. Taking the increase in the RI areas (Figure 8) into account shows the ICUMSA data to be reasonable.

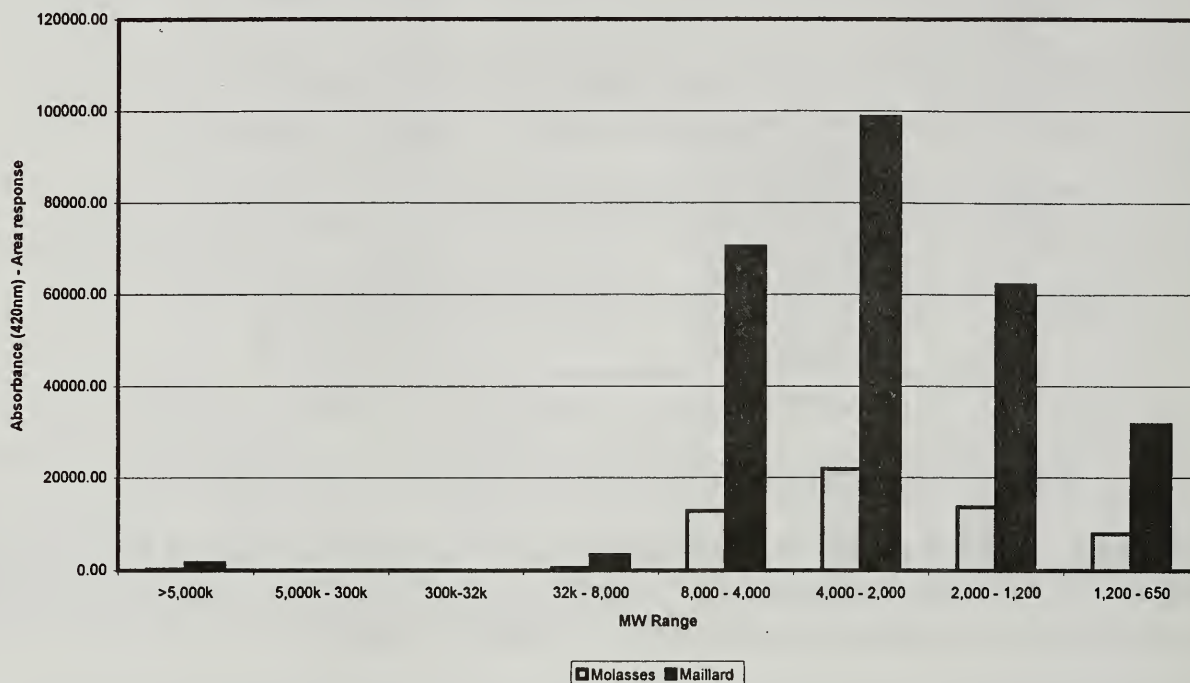


Figure 9. Maillard Reactions - Absorbance at 420nm Response

Cane Juice and Iron

It is well established that enzymes play an important role in the formation of color (Coombs & Baldry, 1978). Before these enzymes are denatured, they can form significant amounts of color. Iron also is implicated in the mechanisms of color formation. Godshall (2000) reports that the ferrous iron (Fe^{2+}) can form complexes with phenolics and caramels to form darker products. To investigate these effects three experiments were performed.

1. Untreated cane juice was exposed to iron – enzymes still active
2. Cane juice was autoclaved before exposure to iron – thermally sterilized
3. Cane juice treated with mercuric chloride (HgCl_2) – enzymes chemically denatured

Untreated cane juice shows small but significant increases in color when heated (Figure 11). The samples exposed to iron show a similar behavior (add or subtract 5 units) except in the 7,500 to 4,000 MW range where a large jump in color is seen relative to the initial juice and the control experiment. The changes in concentration are however too small to be significant (Figure 10). This implies that color formation occurs without forming compounds of a different molecular weight. For the remainder of this analysis the RI data will not be included.

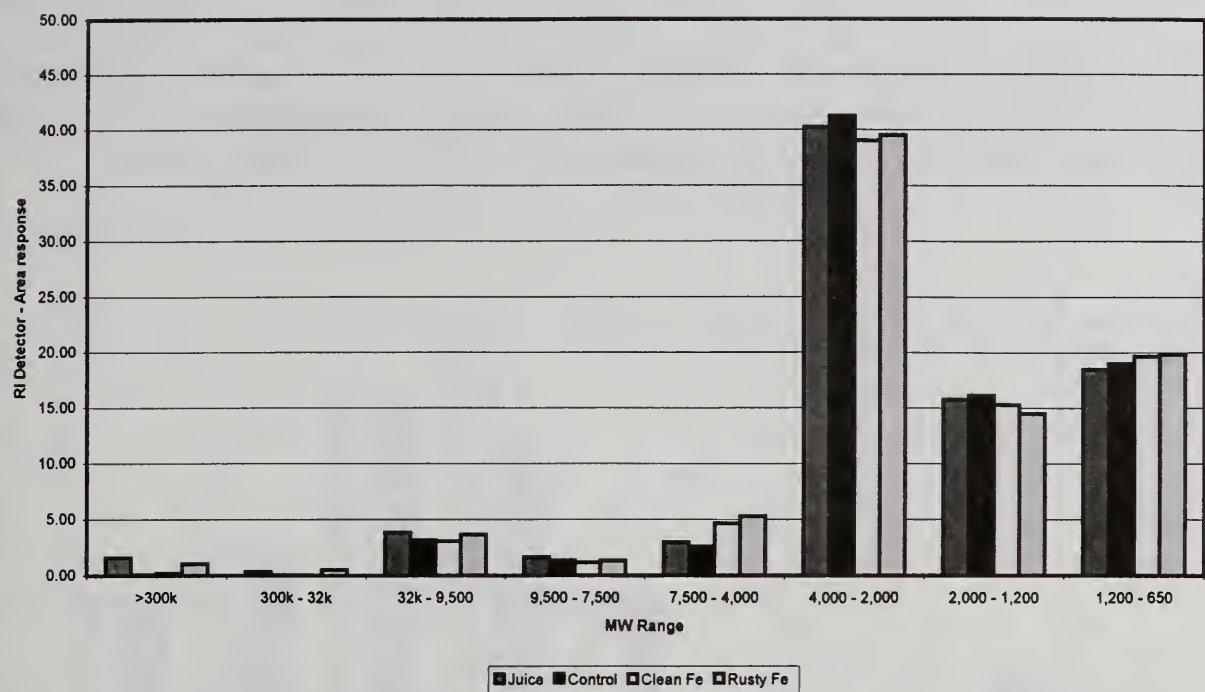


Figure 10. The effect of iron on untreated cane juice – RI Area Response

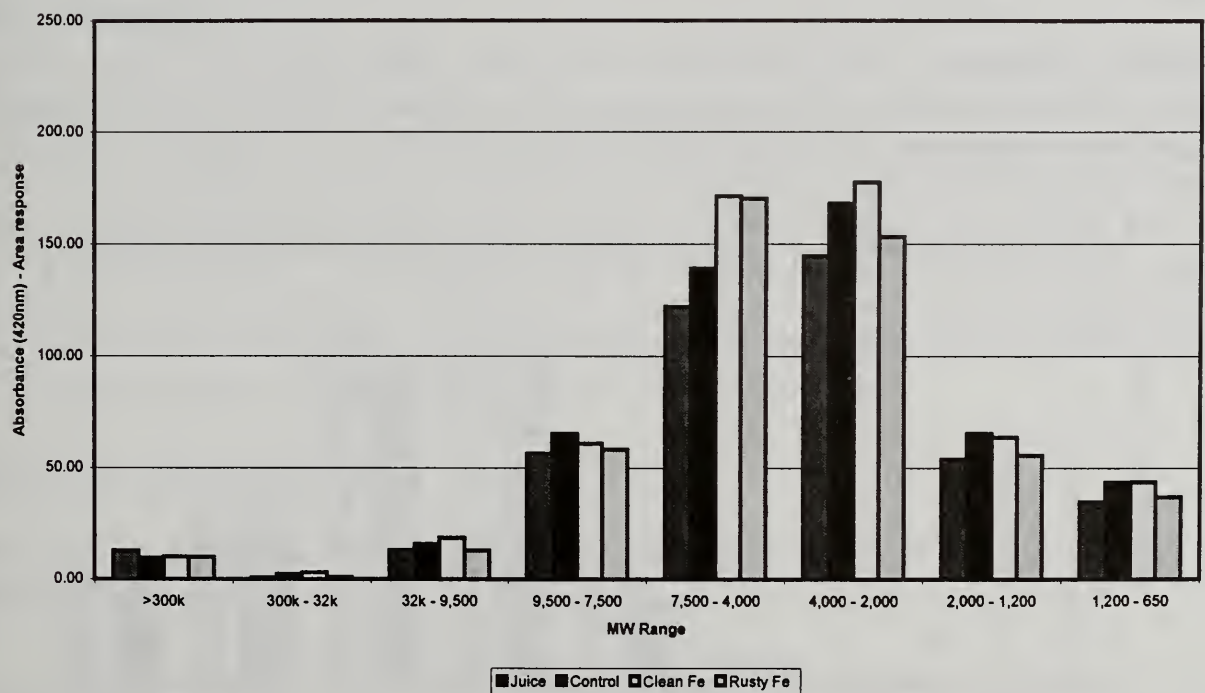


Figure 11. The effect of iron on untreated cane juice – ABS (420nm) Area Response

Autoclaved juice that is exposed to iron also shows the increase in color in the 7,500 to 4,000 MW range. The other ranges show either no change or a slight decrease in color. The control experiment shows only a small change in this range and so the effect seen is the action of iron.

This suggests that color formation in the presence of iron leads to a colorant of a specific molecular weight (7,500 to 4,000 MW) and that enzymes form relatively small amounts of colorant in all ranges less than 300k. To confirm this conclusion a second test was performed. If after denaturing the enzymes with mercuric chloride, cane juice produces colorant in the 7,500 to 4,000MW range, this must be due to the formation of colorant by the action of iron.

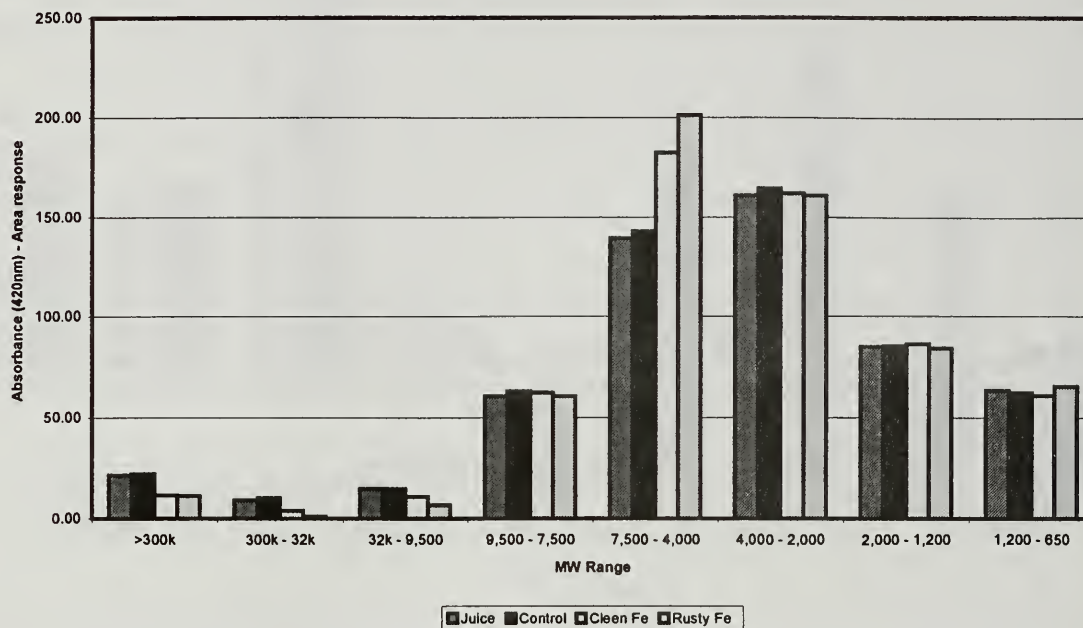


Figure 12. The effect of iron on autoclaved cane juice – ABS (420nm) Area Response

The addition of mercuric chloride showed a very similar effect (Figure 13). The only major increase in color is observed in the same range, confirming the conclusion. No conclusion can be drawn from comparing the effects of rusty and clean iron.

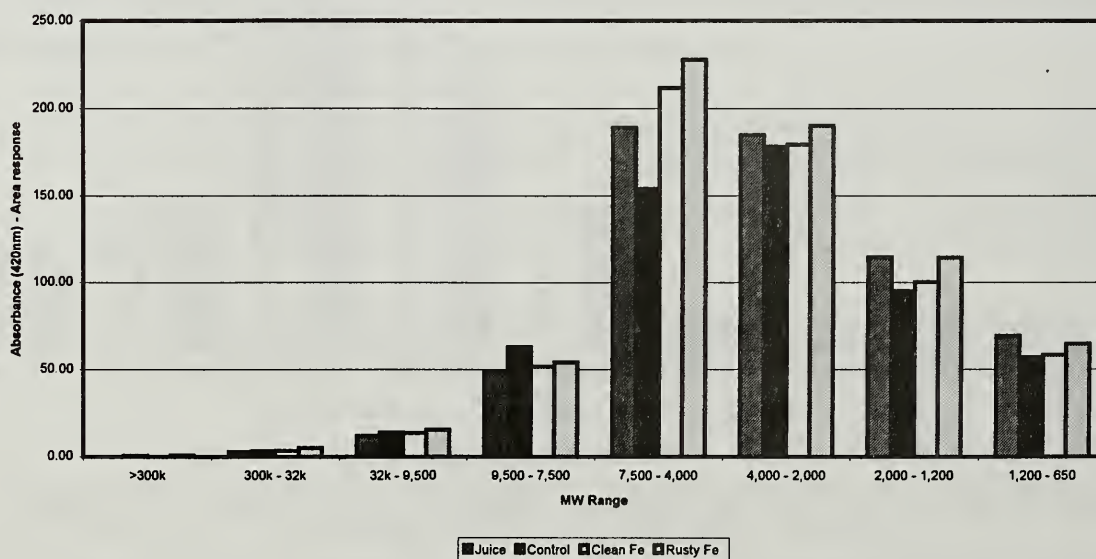


Figure 13. The effect of iron on cane juice with 1:5000 part Mercuric Chloride– ABS (420nm) Area Response

MALDI-TOF-MS

In the past, Gas Chromatography-Mass Spectrometry has been used for identification of color compounds. This is somewhat limiting as only compounds less than about 1,000MW can be identified, as higher molecular weight compounds will not volatilize in the column (Godshall, 1996). Sucrose is also a major concern and must be removed from the sample (Letcher & Whitehead, 1996).

Matrix-assisted laser desorption/ionization time-of-flight mass-spectroscopy is capable of ionizing compounds of molecular weights up to about 100,000 MW as laser radiation is used, making the system far more suited to the identification of color compounds. Information about the structure of the compound being analyzed includes repeat unit molecular weight and end-group molecular weight. In general, MALDI gives either good results or none at all. A major problem is the removal of all salts from the samples as they mask the detector. Dialysis is a simple solution to the problem. More complicated though is picking the correct matrix to use. A matrix compound is used to stabilize the analyte as it ionizes.

HABA {2-(4-Hydroxyphenylazo)benzoic acid} was picked as the matrix as it works with a wide range compounds. Unfortunately, only response for the matrix was observed. Hao et al (1998) report successfully analyzing dextrans using DHB (2,5-dihydroxybenzoic acid) as the matrix. This will be a good place to begin future research.

CONCLUSIONS

GPC has proved to be a reliable method in characterizing the concentration and color of material with molecular weight 650 and above. The majority of cane sugar colorants in the sugar mill have a molecular weight less than 10,000. In caramelization, alkaline degradation and the Maillard reactions, material of molecular weight less than 650 is polymerized into higher molecular weight material. In these reactions the products all fall within the same molecular weight ranges. This is particularly significant for modeling factory processes as each band may be used as a separate component. Cane juice contains enzymes that form colored material. If juice is exposed to iron, color forms in the 7,500 to 4,000 MW range.

ACKNOWLEDGEMENTS

S Ryan, a Ph.D. student from the Department of Chemistry, Louisiana State University, for his assistance in FTIR studies.

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APPENDIX

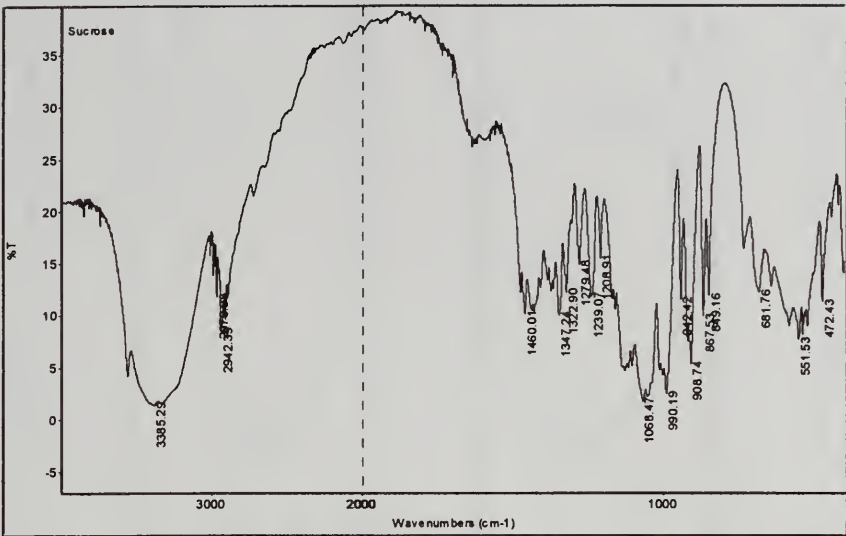


Figure A.1. FTIR Spectrum of Sucrose

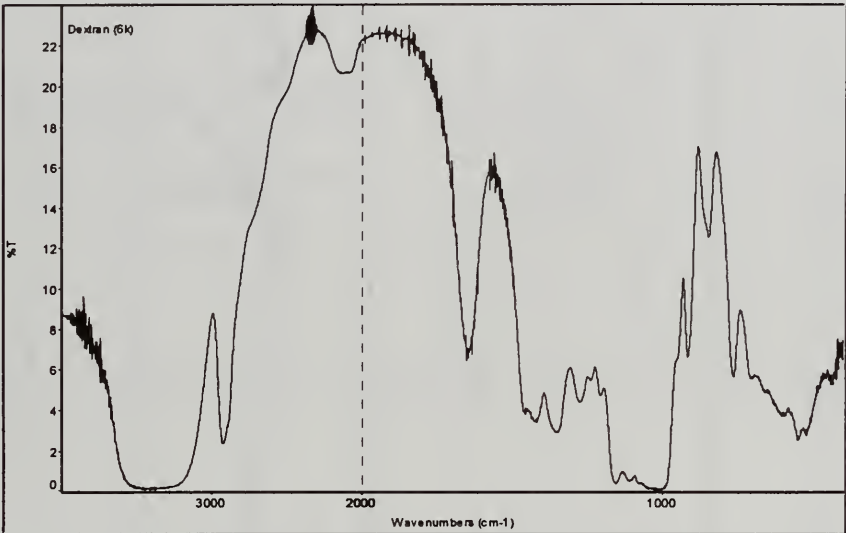


Figure A.2. FTIR Spectrum of 6,000 MW Dextran (from *Leuconostoc mesenteroides*)

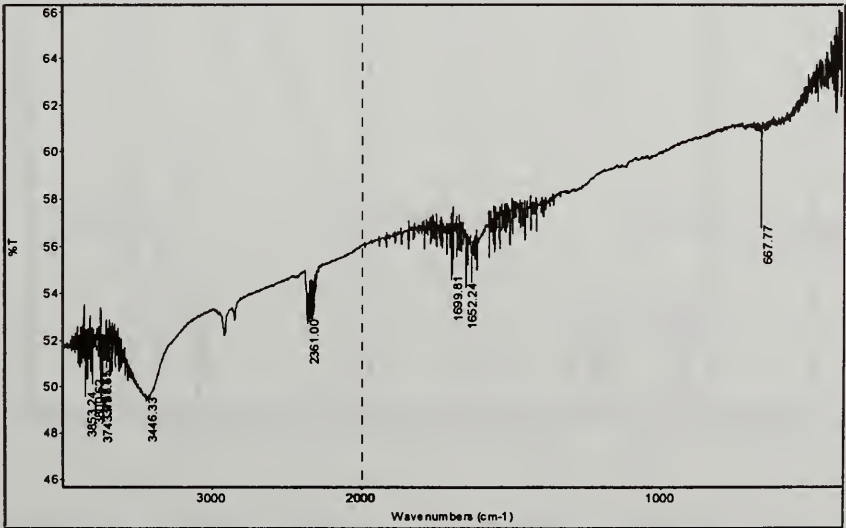


Figure A.3. FTIR Background Spectrum – Potassium Bromide

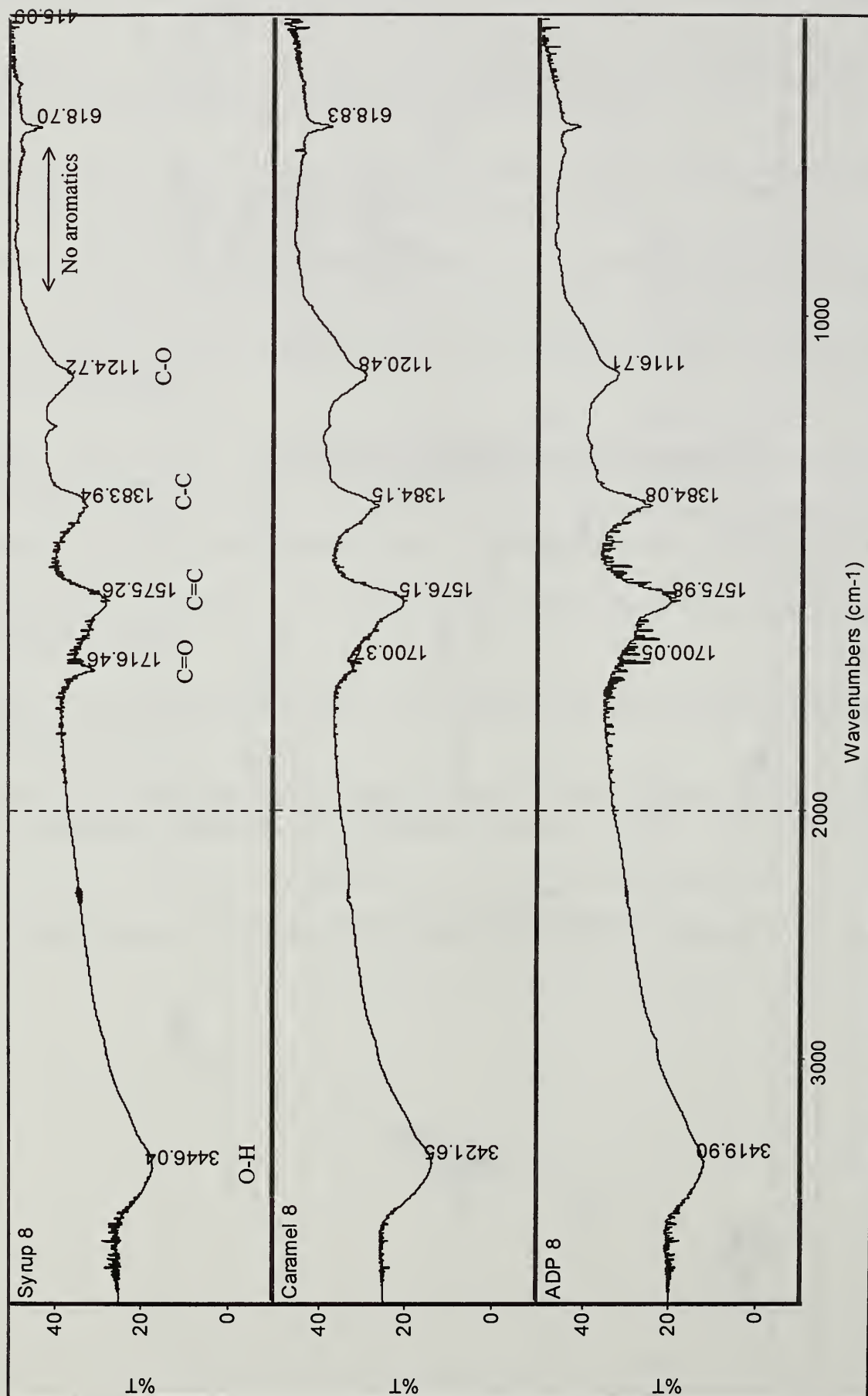


Figure A.4. FTIR Spectra of Caramelization and alkaline degradation: 1,200-650 MW

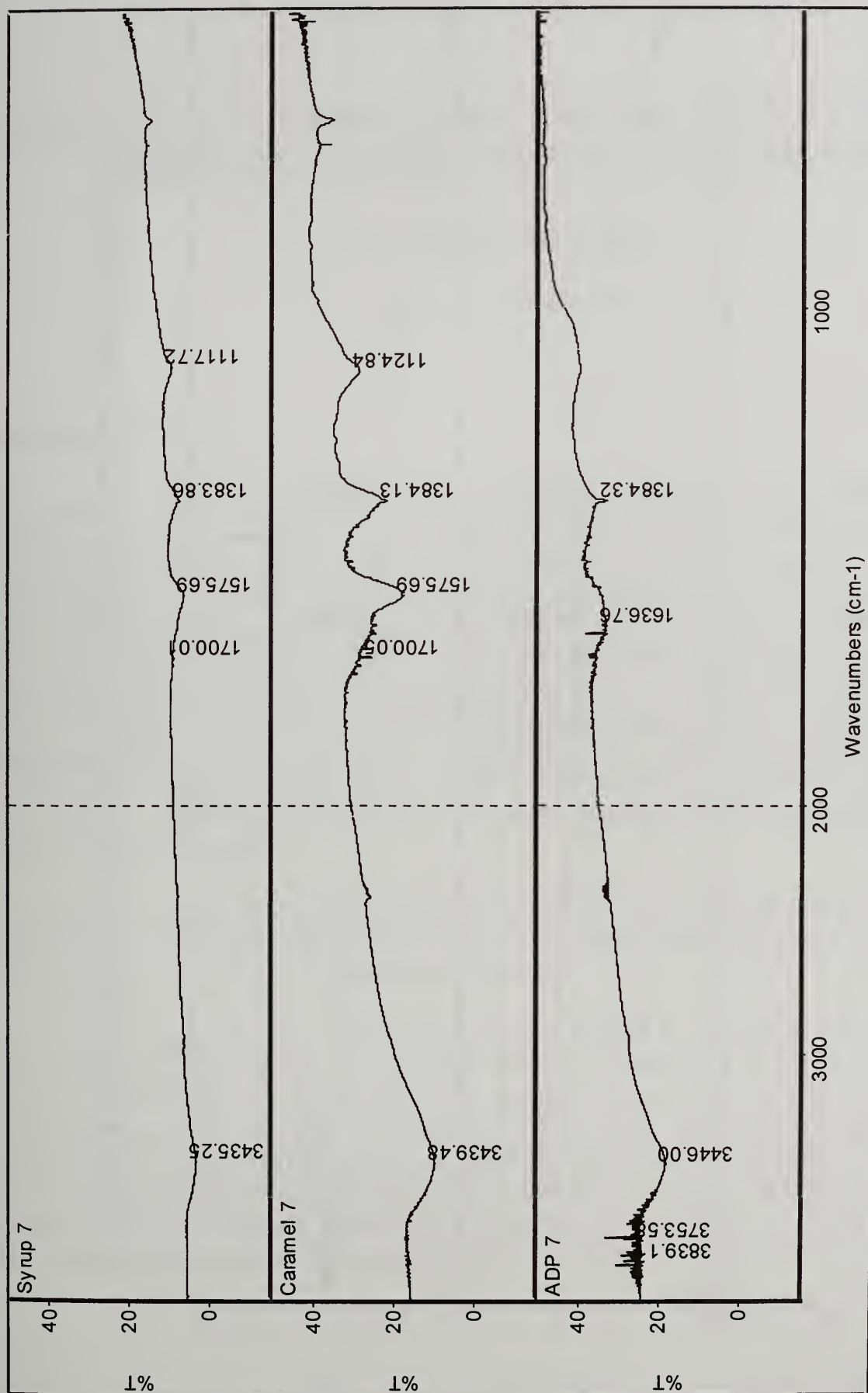


Figure A.5. FTIR Spectra of Caramelization and alkaline degradation: 2,000-1,200 MW

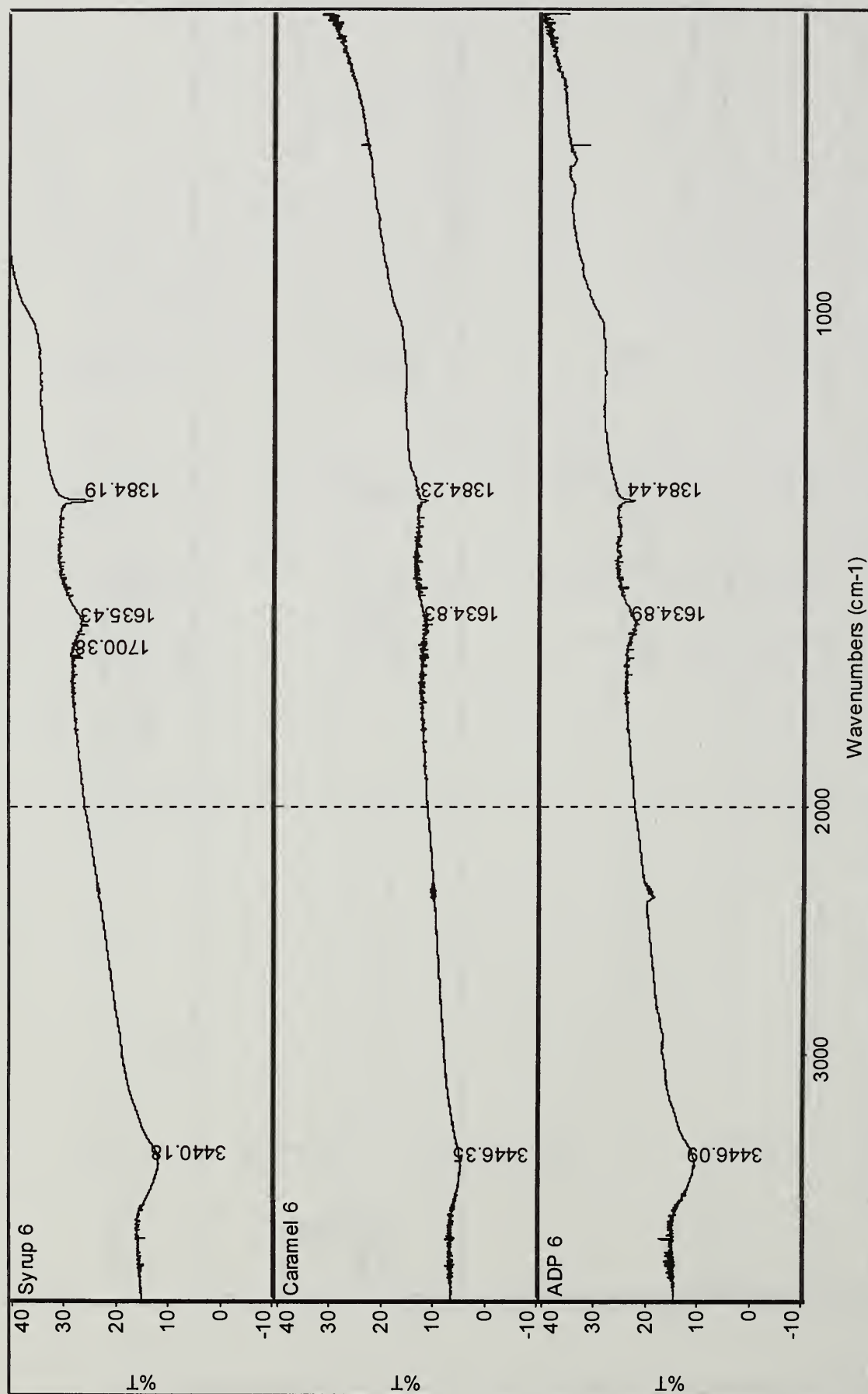


Figure A.6: FTIR Spectra of Caramelization and alkaline degradation: 4,000-2,000 MW

SEPARATION OF BEET AND CANE SUGAR COLORANTS THROUGH STYRENIC STRONG BASE RESINS

Luis San Miguel Bento

CIENTECA - Portugal

INTRODUCTION

Color in sugar is due to a complex mixture of compounds of different nature. These compounds can originate from the cane plant or can be formed during the extraction and refining process. A great many studies have been made to try to identify or study the chemical nature of these compounds: their molecular weight, ionic charge and hydrophobicity /hydrophilicity. Other studies were made to identify individual components (Farber and Carpenter, 1971; Paton *et al.*, 1985; Smith, 1976). Molecular weight of such compounds was done using GPC (gel permeation chromatography) using UV and RI detectors (Godshall, *et al.*, 1988a, 1988b) and ELS detectors (Bento, 1997). Another way to evaluate the predominant nature of color components is through the indicator effect, as the IV - indicator value (Clarke, *et al.*, 1987). This value is determined as the quotient of the absorbancy at 420 nm at pH 9.0 and at pH 4.0. Higher IV values indicate a predominance of cane pigment colorants and low IV indicates the predominance of colorants produced during processing.

In this study we have separated sugar colorants contained in raw sugars from cane and beet origin by fixing them onto a strong base styrenic resin. Resin regeneration was accomplished by increasing sodium chloride concentration and alkalinity.

The fractions separated during regeneration were evaluated by analyzing color, IV, and UV spectrophotometry. The spectra of individual fractions at different pH (pH 9 and pH 3) were analyzed and compared. In the spectral zone between 250 and 310 nm some fractions present higher absorbancies at low pH. In order to enhance these differences of absorbencies, the quotient (ratio) between the absorbances at pH 9 and pH 3 was plotted against the wavelength. These values were referred to as the UIV (Ultraviolet IV). A study of the behavior of UIV in the different colorant fraction was undertaken. Comparison of the UIV of colorants in washed raw sugar, colorant fractions, phenolic acids, fine liquor and white sugar, was done.

MATERIALS AND METHODS

Resin Preparation

Resin used for this work was a styrenic divinylbenzene resin, IRA 900C Cl. Resin was placed in a water-jacketed column (Pharmacia and Kontes). Water at 60° C was circulated through the column external wall, during all the steps of this work. Before the resin was used it was washed in the following way:

- distilled water (10 BV at 5 BV/h)
- NaCl 2M + NaOH 1.5% (30 BV at 3 BV/h)
- 2/3 NaCl 3M + 1/3 Methanol + NaOH 1.5% (10 BV at 3 BV/h)
- NaCl 2M (10 BV at 3 BV/h)
- distilled water (20 BV at 5 BV/h)
-

Wash water and regenerants were heated at 60° C. Methanol mixture was heated at 50°C.

After each step, with regenerant, eluent UV spectra were determined with regenerant as the blank. The objective was to have an effluent in each step with absorbancies through all the spectra with values lower than 0.100, using a 1 cm cell.

Feed Liquor Preparation and Charge

Three runs were made using beet and cane raw sugar to prepare feed liquors. The objective was to charge the resin with 12,000 kg DM * IU color / liter of resin. This value is lower than the resin capacity. The intention was to fix the maximum amount of colorants to the resin, avoiding leakage of colorants to the fine liquor. Conditions of resin charge are presented at Table 1.

Table 1. Resin charge

Run #	Raw Sugar			Raw Liquor		Resin	Color charged*
	Type	Color	Quantity	Volume	Brix	Volume	
1	Beet	5283 IU	872 g	4000 ml	20° Bx	400 ml	11,459
2	Cane I	1957 IU	2350 g	5000 ml	40° Bx	400 ml	11,461
3	Cane II	4563 IU	390 g	1800 ml	20°Bx	150 ml	11,830

* kg MS * UI/l of resin

Feed liquor was prepared by dissolving cane and beet raw sugars in distilled water. The solution was then filtered through filter paper Whatman 41 with filter aid. Filtrate was then alkalized to 8.5±0.1 with NaOH. Feed solution was heated to 60°C before be passed through the resin at a flow of 3 BV/h.

Resin Regeneration

Before regeneration, the resin was washed with distilled water for f 20 BV at a flow rate of 5 BV/h. Resin was then regenerated with sodium chloride at increasing concentrations and alkalinities (Table 2).

Table 2. Composition of Regenerants

Step	NaCl	NaOH	Methanol
A	0.2 M	-	-
B	0.4 M	-	-
C	0.6 M	-	-
D	2.0 M	-	-
E	2.0 M	0.5 %	-
F	2.0 M	1.0 %	-
G	2.0 M *	1.5 %	33.3 % v/v

* in Run 3 a concentration of 1.0 M of NaCl was used.

Regenerants were fed to the resin at a flow of 3 BV/h and at a temperature of 60°C, except regenerant G which was heated to 50° C. After each regeneration step, the resin column was emptied of liquid, in order to separate different regenerants. A volume of 1200 ml of regenerant, 3 BV, in each step was used in runs 1 and 2. In these runs an intermediate wash with 500ml of water was done between each step. In run 3, a volume of 900 ml of regenerant, 6 BV, was used without any intermediate wash. Eluents resulting from each regeneration were collected separately, and are named here as Fraction A, B, etc.

Spectrophotometric Analysis

Samples were analyzed by spectrophotometry in a 1cm cell length at wavelengths between 220 and 450 nm with a Perkin Elmer Lambda 11 spectrophotometer. The blank was a solution of NaCl 5.0 g; methanol 20.0 ml; extra white sugar 5.0 g with distilled water to 100 ml.

Each fraction was diluted with distilled water to have an absorbancy of 0.500 ± 0.100 , at 270 nm, with a 1 cm cell. In order to have the same composition in each sample, chemicals were added in a quantity to have a final composition of 5 g NaCl, 20 ml of methanol and 5 g of sugar to 100ml in each sample (Table 3). Distilled water was added to make up the final volume. NaCl added compensate for the salt in solution and salt formed during neutralization of NaOH. Samples alkalized with NaOH were mixed with HCl in order to have a pH near neutrality in order to make the pH correction easier.

Solutions were filtered through 1.2 μ m filter under vacuum. Aliquots of filtrate were treated with NaOH or HCl in order to have a final pH of 9.00 ± 0.05 and 3.00 ± 0.05 . The final volume was maintained the same in each sample by adding distilled water. The final Brix of each sample was measured by refractometry. Samples of each fraction (pH 9 and pH 3) must have a Brix difference no higher than 0.1° Bx.

Table 3. Composition of each sample for 100 ml

Sample	Effluent ml	Added chemicals			
		NaCl g	MtOH ml	HCl ml	Sugar g
A	31.0	4.64	20.0	-	5.0
B	26.0	4.39	20.0	-	5.0
C	31.0	3.78	20.0	-	5.0
D	18.0	2.90	20.0	-	5.0
E	11.5	3.66	20.0	-	5.0
F	44	-	20.0	3.0 (2M)	5.0
G	51	0.85	3.0	4.0 (5M)	5.0

RESULTS AND DISCUSSION

Fractional Regeneration

The majority of sugar colorants have an anionic charge and are amphiphilic in character. Due to these features, colorants are fixed to resins by ionic bonds with the ionic active group, and by hydrophobic interaction, with the resin matrix (Bento, 1992). When a salt solution is passed through the resin, during regeneration, colorants are released according to their affinity and their fixation to the resin. At low salt concentrations colorants with low anionic charge are released (Bento, 1989). At high salt concentrations colorants strongly fixed ionically to the resin, are released. Some colorants can be fixed with both mechanisms (Williams and Bhardwaj, 1992). When these compounds have a high pK they are released only with a high regenerant pH. Other colorants are so tightly fixed to the resin matrix that only with an organic solvent can they be released. By doing a fractional regeneration, it is possible to divide the majority of the colorants in several defined groups.

In this study, a comparison between beet and cane raw sugar colorants was done with 400 ml of resin in a Kontes column (Runs 1 and 2). Run 3 was done with 150 ml of resin in a Pharmacia column. Each Fraction was analyzed for absorbancies at 420 nm, pH 9.0, and IV. Results are presented in Table 4 and in Figures 1 and 2.

Table 4 – Analysis of each Fraction

	Beet Raw Sugar			Cane Raw Sugar (I)			Cane Raw Sugar (II)	
	Abs. *	IV	PH	Abs. *	IV	pH	Abs. *	IV
A	0.147	1.24	8.49	0.263	2.30	7.08	0.062	2.05
B	0.223	1.31	9.09	0.337	1.92	8.59	0.135	2.14
C	0.315	1.20	8.67	0.439	1.70	8.32	0.209	1.50
D	1.035	1.12	8.06	1.084	1.65	8.30	0.595	1.40
E	0.294	1.24	12.19	1.088	1.94	12.49	1.218	1.92
F	0.095	1.25	12.39	0.285	2.30	12.78	0.366	2.45
G	0.137	1.05	12.79	0.588	3.00	12.90	0.348	3.87

* at 420nm, pH 9, equivalent to 3 BV

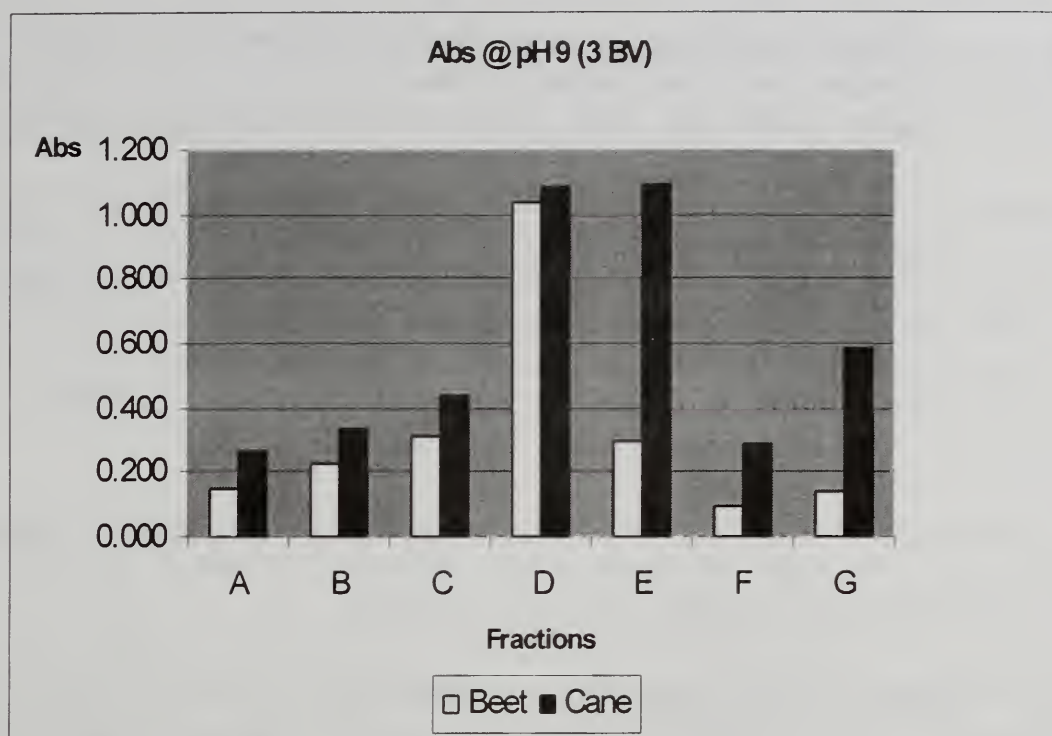


Figure 1 – Absorbancy of Beet and Cane Fractions

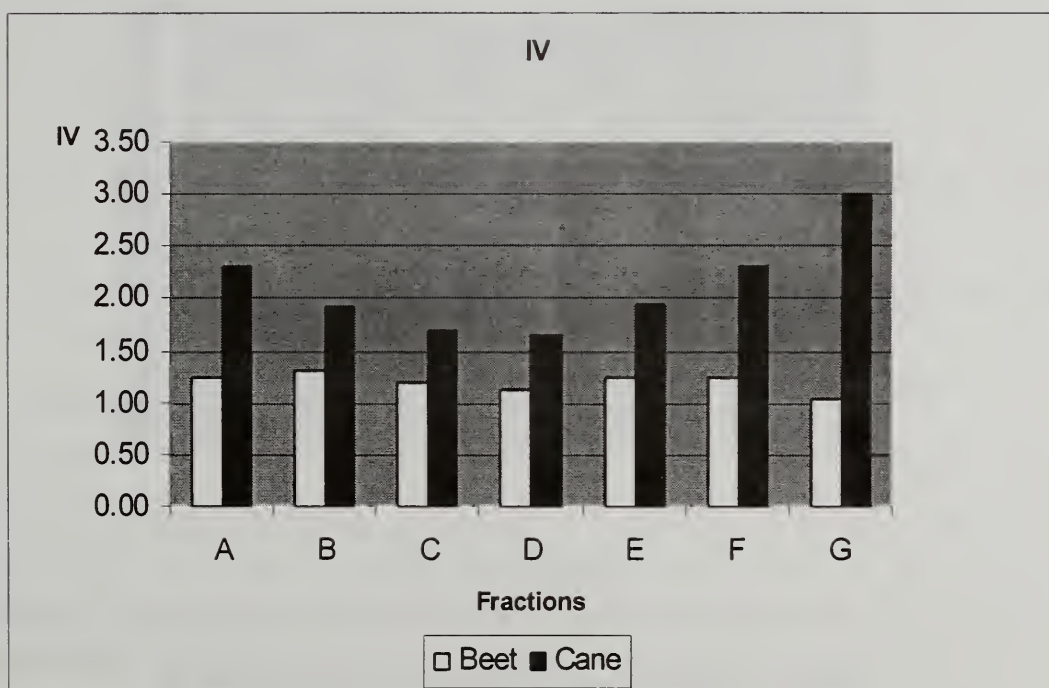


Figure 2 – IV of Beet and Cane Fractions

It was observed that beet colorants were mostly released in Fraction D (46%). This was observed during the colorant charge in the resin. Beet colorants remained in a narrow zone at the top of the resin. With cane colorants, there was a spread of colorant during the entire cycle. This means that beet colorants are mainly anionically charged and fixed to the resin by this mechanism.

Cane colorants are mainly released in Fractions D and E (26% each). This means that these colorants have an anionic charge and part of them must have a high pH to be released from the resin. This means that they must be fixed hydrophobically under process conditions. They need to be at a higher pH in order to be in an ionic form and be released from the resin. In cane, it is also observed that some colorants (14%) are released in Fraction G (more than in beet). These colorants can switch from one to the other type of fixation to the resin (Bento, 1992) and are irreversibly fixed to the resin when salt regeneration is used.

The IV of each fraction for beet and cane samples is presented in Figure 2. As noted, the IV from beet samples does not vary very much (values between 1.05 and 1.31). This indicates the presence, mainly, of factory colorants in the beet raw sugar.

Referring to the cane raw sugar sample (Run 2) the values vary between 1.65 (fraction D) and 3.00 (fraction G). This indicates that the more charged colorants would be mainly factory colorants, probably melanoidins. In Run 3 (cane sugar) these results were confirmed (Figure 3). As IV increased when a raw sugar is affined, we can suppose that colorants inside the crystal are the ones in fractions A, B, E, F or G (in one or various fractions).

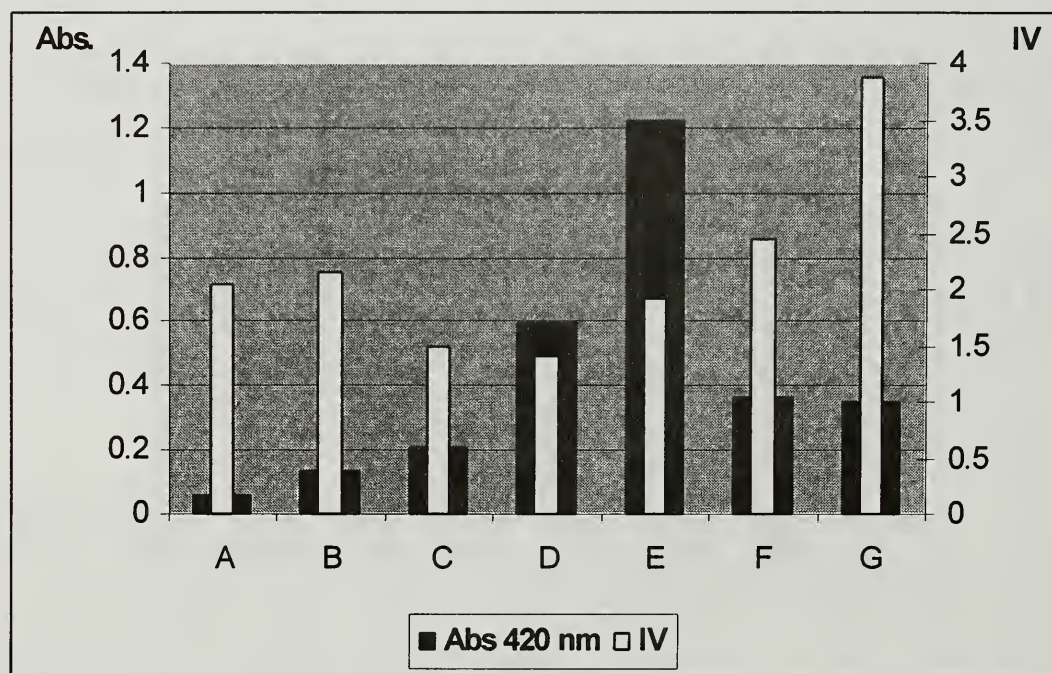


Figure 3 – Color and IV for Cane Sugar (run 3)

The percentage of color of each fraction in the three runs is presented in Table 5. As some colorants were passed through the resin to the Fine Liquor, some of these colorants have a higher percentage in the original raw sugar.

Table 5 – Percentage of colorants (Abs at 420 nm at pH 9)

Fraction	Beet Raw Sugar	Cane Raw Sugar (I)	Cane Raw Sugar (II)
A	6.5	6.4	2.1
B	9.9	8.2	4.6
C	14.0	10.7	7.1
D	46.1	26.5	20.3
E	13.1	26.6	41.5
F	4.2	7.0	12.5
G	6.1	14.4	11.9

Spectrophotometric studies

Samples corresponding to each fraction of cane raw sugar (Run 3) were studied by spectrophotometry. Spectra of fractions A to G are presented in Figures 14 to 20 (Appendix). Analyzing the spectra, it is observed that absorbances of samples at pH 9 are higher than samples at pH 3, except in the range 254 to 306 nm and under 226 nm in Fraction A; 264 to 302 nm and under 240 nm in Fraction B; 261 to 292 nm and under 229 nm in Fraction C; 264 to 304 nm in Fraction D; and in the range of 221 to 228 nm and over 290 nm in Fraction G. In Fraction E and F all the absorbances of pH 9 samples are higher than the ones of pH 3 samples.

To enhance these differences in absorbance at pH 9 and pH 3 we plotted the UIV function (UIV – Ultraviolet IV), defined by:

$$\text{UIV} = \text{Abs}(\lambda) \text{ at pH 9} / \text{Abs}(\lambda) \text{ at pH 3.}$$

With wavelengths, λ , varying from 220 to 320 nm. Figures 4 and 5 show the UIV of Fractions A to G.

It is observed that each fraction presents a different UIV plot. The minima and maxims of the UIV curves of each fraction are (UIV values are presented in brackets):

Fraction A presents minima at 266 nm (0.95) and 294 nm (0.95).

Fraction B presents a minimum at 285 nm (0.95).

Fraction C presents a minimum at 268 nm (0.98).

Fraction D presents a minimum at 294 nm (0.99).

Fraction E presents a maximum at 282 nm (1.07).

Fraction F presents a maximum at 283 nm (1.09).

Fraction G presents a maximum at 281 nm (1.04).

The peaks (maxima) between 241 and 249 nm with UIV values from 1.04 to 1.11 were not interpreted in this study.

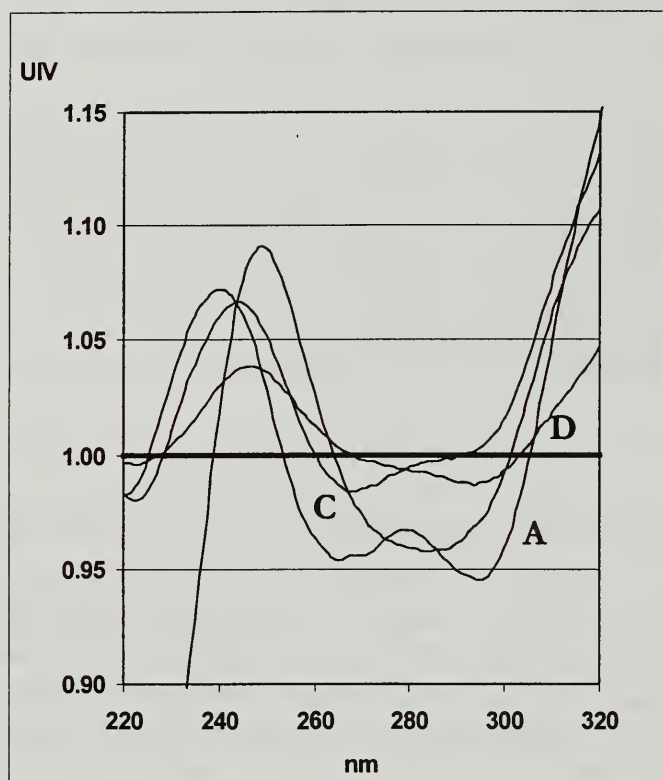


Figure 4 – UV of Fractions A, B, C, D

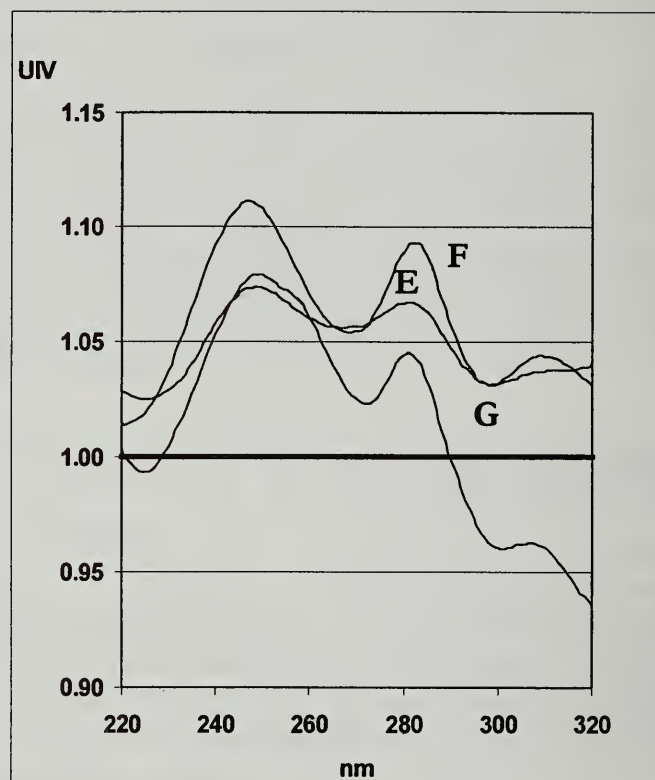


Figure 5 – UV of Fractions E, F, G

To interpret these results, UV values of synthetic colorants and phenolic acids were determined.

UV of synthetic colorants

Synthetic colorants were prepared according to Shore, *et al.* (1984). UV values were determined in the same way as eluent fractions described earlier. Results are presented in Figures 6 and 7. In Figures 8 and 9 is presented the UV of two phenolic acids.

These curves present the following minima and maxima:

- Melanoidin presents a minimum at 291 nm (0.98)
- HADP presents a minimum at 262 nm (0.82).
- Ferulic Acid presents a maximum at 262 nm (1.45)
- Caffeic Acid presents a maximum at 263 nm (2.02).

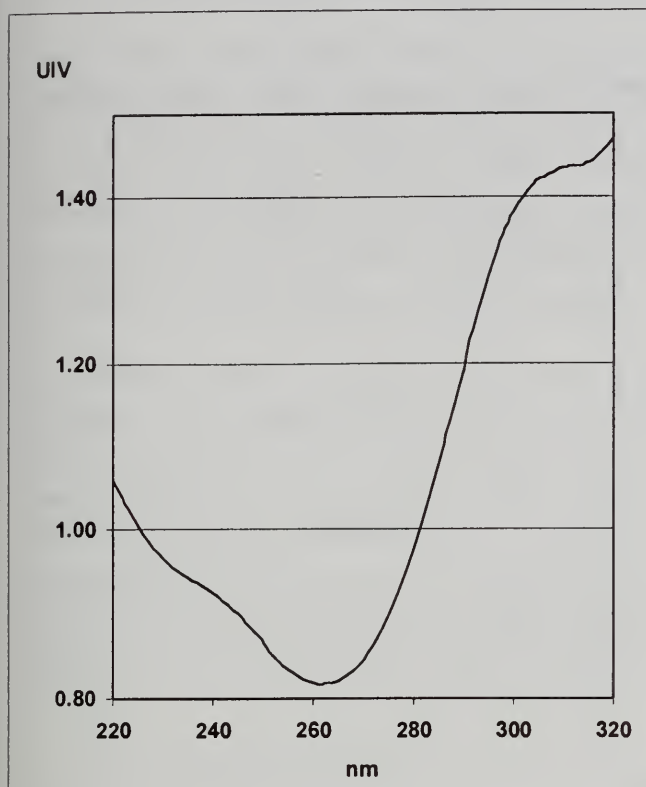


Figure 6 – UIV of Melanoidin.

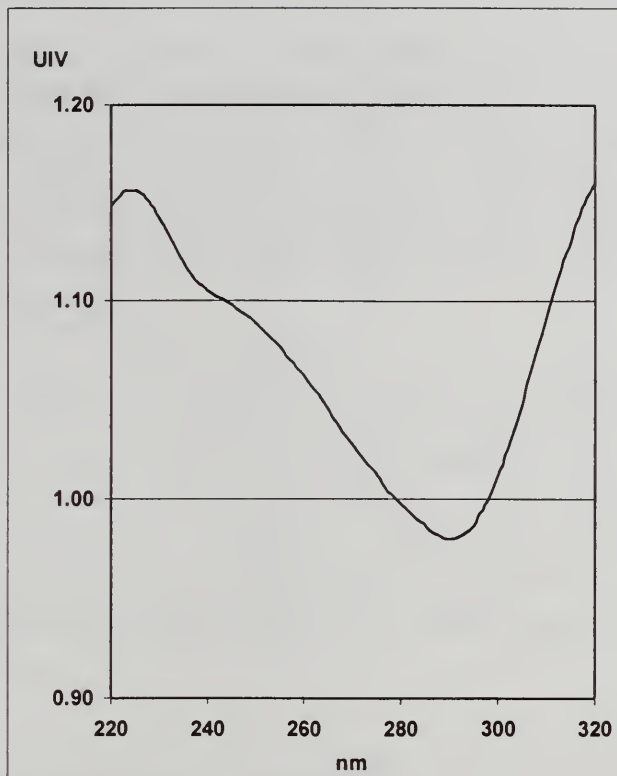


Figure 7 – UIV of HADP.

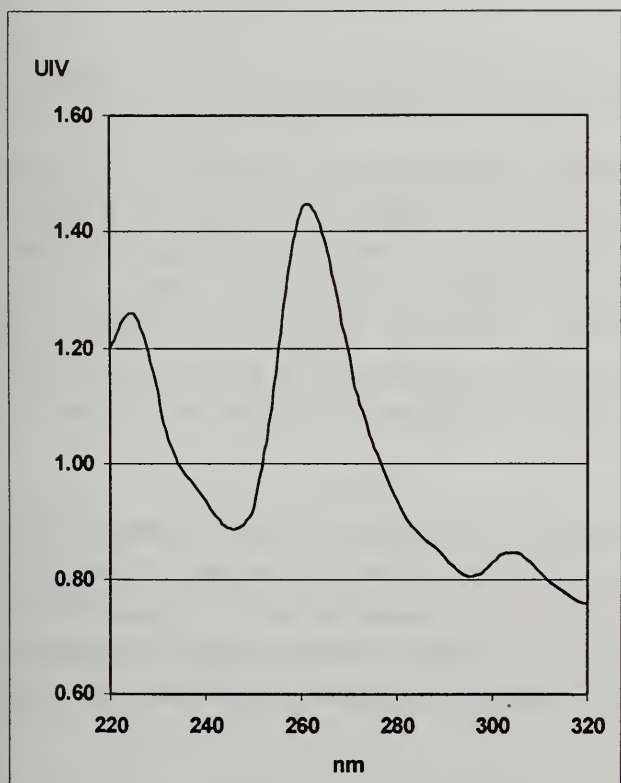


Figure 8 – UIV of Ferulic Acid.

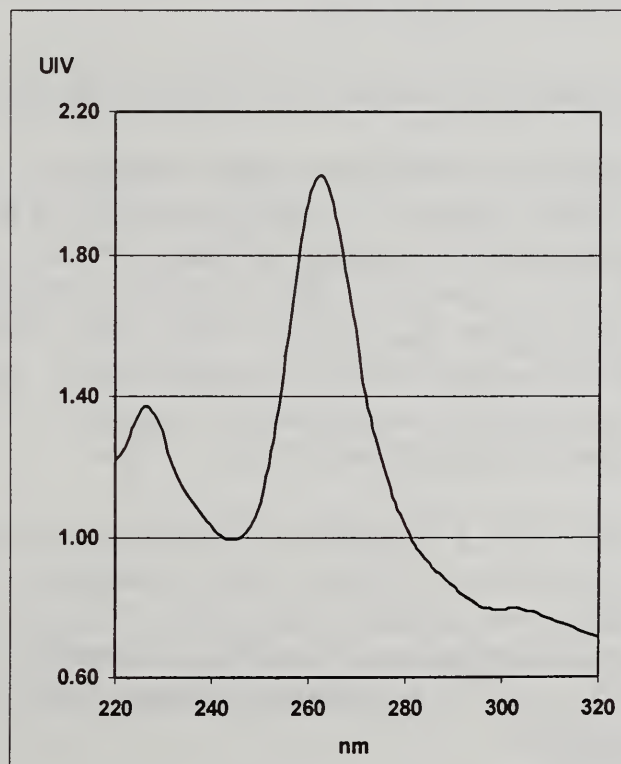


Figure 9 – UIV of Caffeic Acid.

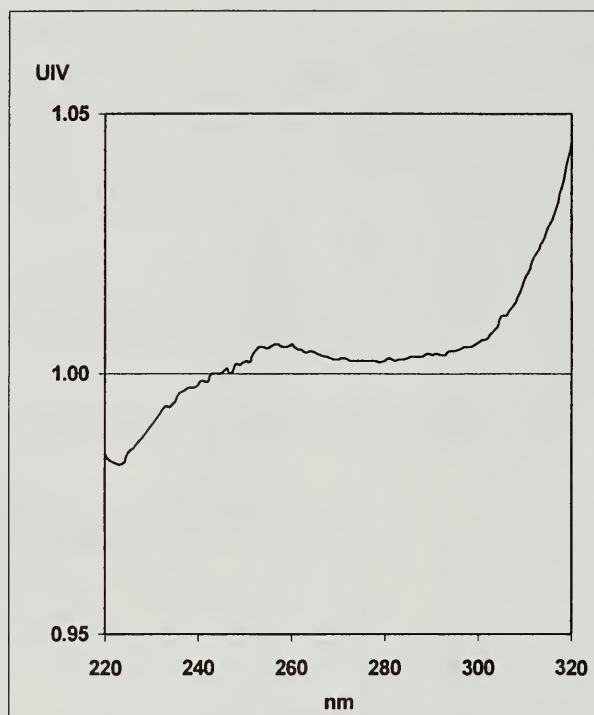


Figure 10 – UV of Caramel

Caramels do not present a great variation with pH, having UV values close to one between 240 and 300 nm (Figure 10).

Comparing these results with the ones obtained with the regeneration fractions we can conclude:

Fraction A presents two peaks corresponding to ADPH (266 nm) and melanoidin (294 nm). Fraction B presents a peak in the middle of the two preceding values (268 nm) being, probably another colorant group, not analyzed here, as products of enzymatic browning reactions. Fraction C presents a peak (268 nm) corresponding to ADPH but with a UV value near one. This suggests that in this fraction may exist a mixture of ADPH and caramel. Fraction D presents a peak (294 nm) corresponding to melanoidins. These melanoidins must have a higher charge than the ones released in Fraction A. In fact, the term melanoidins refers to a mixture of colorants with different chemical nature.

Fractions E, F and G may contain phenolic compounds. It was observed that Fraction G, after contact with air, formed a bulky precipitate. In Fraction G, UV curves in the range higher than 290 nm were similar to the ferulic Acid curve. Ferulic acid is associated with polysaccharides, normally being esterified with arabinoxylan (Clarke, *et al.*, 1988). In a GPC study (Vercellotti, 2001) it was detected that Fractions E to G, presented a higher molecular weight than the other fractions.

UIV of Raw Sugar, Washed Raw Sugar, Fine Liquor and White Sugar

Figure shows the UIV curve of Fine Liquor (output of resin at run 3) being the upper curve corresponding to the first bed volume of liquor and the lowest curve corresponding to the 10th BV of Fine Liquor. All the UIV values present a minimum peak at 282 nm with UIV values varying from 0.99 (1 BV) to 0.92 (10 BV). Possibly, during the resin run, higher charged colorants (with higher UIV values) will dislocate colorants already fixed to the resin, with a low charge (lower UIV values). This leakage of low charged colorants to the Fine Liquor must be considered when making a colorant balance of regeneration.

The UIV of cane raw sugar (run 3) and white sugar are compared in Figure 12. Raw sugar presents two peaks at 274 nm (0.99) and 292nm (0.97). White sugar presents a peak at 272 nm (0.95). Melanoidin has a great predominance in colorants of this raw sugar (peak 292 nm). This peak disappears in the white, sugar meaning that during the refining process melanoidins are removed either in carbonatation or by ion exchange resins. The peak of white sugar (272 nm) approaching ADPH value indicates that this compound is formed during the refining process, mainly in carbonatation (Bento, 1999). Caramels may also be present in this white sugar as UIV value (0.95) is lower that the ADPH value (0.82).

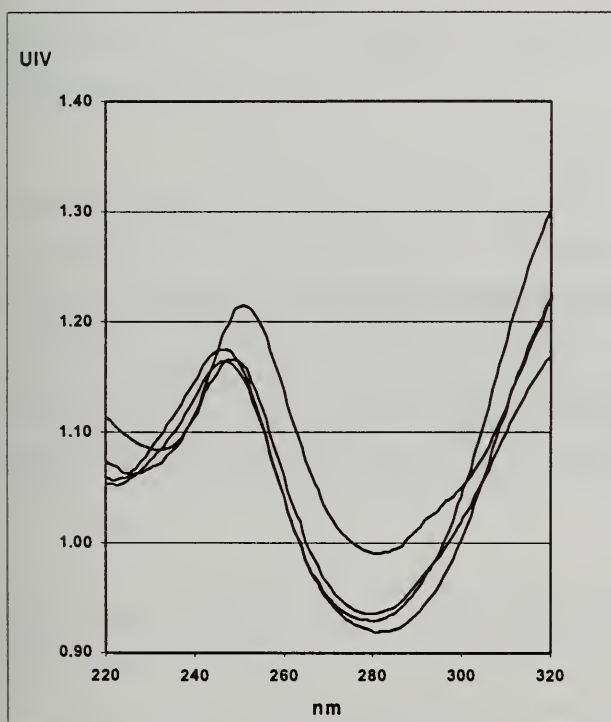


Figure 11 – UIV of Fine Liquor.

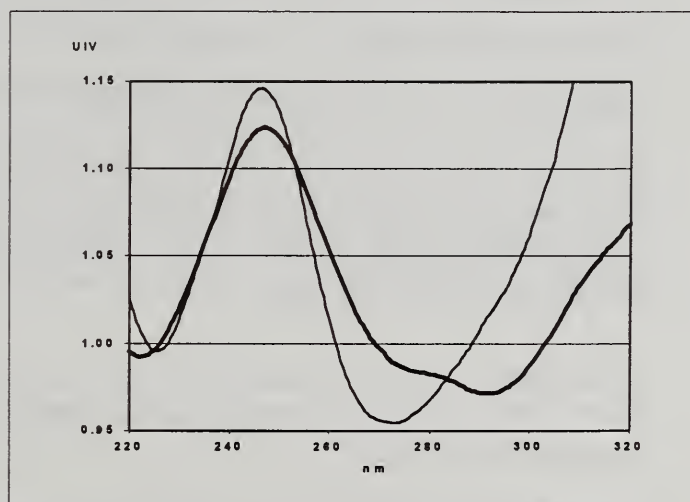


Figure 12 – UIV of Cane Raw Sugar (bold) and White Sugar.

A problem that concerns sugar technologists is to know the characteristics of sugar colorants that are preferentially fixed inside sugar crystals during crystallization. To study colorants inside crystals, cane raw sugar was washed with methanol solution (90:10 methanol:water). The Spectrum and UIV of washed sugar are presented in Figure 13. In this figure is also shown the UIV of a white sugar with 76 ICUMSA color and Fractions A and B.

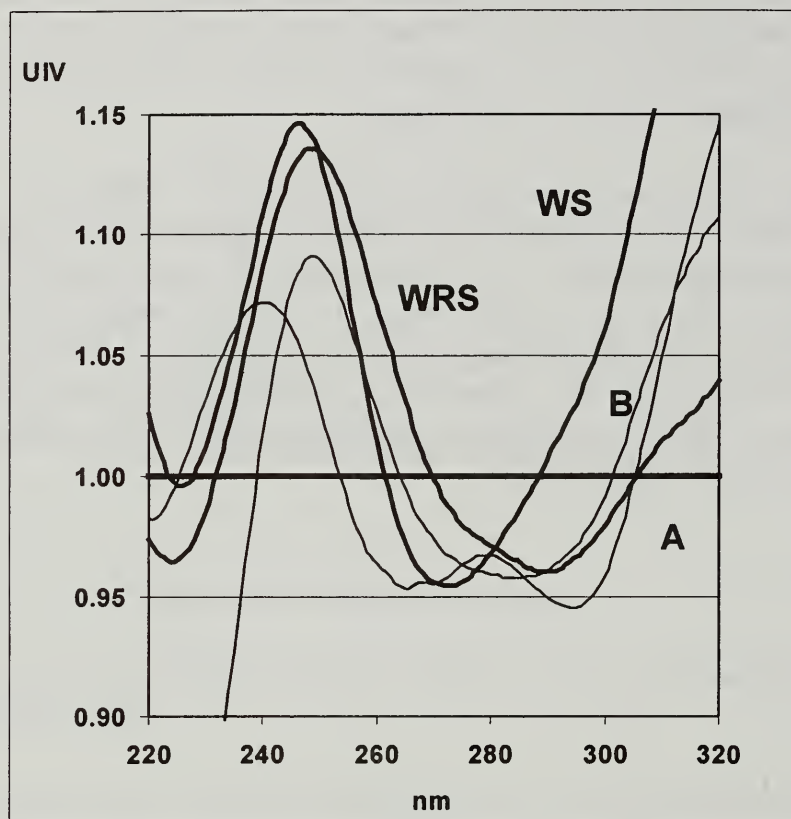


Figure 13 – UIV of Washed Raw Sugar (WRS), White Sugar (WS) and Fractions A and B.

As observed in Figure 13, the UIV of washed raw sugar presents a minimum at 290 nm (0.96). This peak is similar to the second peak of Fraction A (peak 294 nm) corresponding to low charged melanoidins. These colorants probably are removed during the refining process and will be not present in white sugar.

White sugar presents a peak at 272 nm (0.95) similar to the first peak of Fraction A at 266 nm (0.95). This peak may correspond to neutral phenolics that have a high inclusion into sugar crystals (Paton, 1992).

The UIV curve of Fraction B, with a peak at 285 nm (0.95) is also similar to colorants in washed raw sugar with a peak of 290 nm (0.96).

This indicates that colorants inside the sugar crystal are probably, in a major part, of low anionic charge, as colorants in Fractions A and B.

Fractions Balance

A balance was made for Run 3, with cane sugar. This balance was made referred to absorbancies at 273 nm (Bento, 1995). This balance will comprise compounds that are not necessarily colored in the visible spectrum. The balance is presented at Table 6.

Table 6 – Balance of Run 3

	Abs. 273 nm	Dilution	Volume Mass	Abs. Total	% on input
<u>Input</u>					
Raw Sugar	0.4918	80.000	390 g	15344	100.0
<u>Output</u>					
Fraction A	0.4783	3.226	870 ml	1342	8.8
Fraction B	0.4851	3.846	880 ml	1642	10.7
Fraction C	0.5139	3.226	860 ml	1426	9.3
Fraction D	0.5014	5.556	920 ml	2563	16.7
Fraction E	0.5103	8.696	820 ml	3639	23.7
Fraction F	0.4722	2.273	900 ml	966	6.3
Fraction G	0.4883	1.961	810 ml	776	5.0
Total regenerated				12353	80.5
Fine Liquor	0.5899	2.000	1800 ml	2124	13.8
Not regenerated				868	5.7

In this balance it is observed that compound leakage to fine liquor correspond to 13.8% on input compounds in the raw sugar. If these compounds have a low charge, as discussed earlier, the total of low-charge colorants (Fraction A, B and Fine Liquor) amounts to 33.3%. To improve this separation technique, leakage of compounds to fine liquor must be minimized. To obtain this, colorant charge to the resin must be reduced, probably from 12,000 to 4,000 kg DM x IU color / liter of resin. Lower values will give samples with low colorant concentrations (Table 3). In this balance it is observed that 5.7% of compounds remain in the resin, after regeneration. To minimize this fact, another step to remove highly charged and highly hydrophobic colorants must be included.

CONCLUSIONS

The separation of sugar colorants using ion exchange resins is effective. This technique has the advantage of obtaining a great amount of colorants that can be used in another analytical techniques for further identification.

The technique presented in this paper must be improved in order to minimize colorant leakage to fine liquor and colorants remaining in resin after regeneration. The calculation of UIV (Ultraviolet Indicator Value) can give a good indication of colorant type in a sugar solution. This technique showed that the majority of colorants in sugar crystals have a low anionic charge. This technique can also be used to identify colorant change in each process step.

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APPENDIX

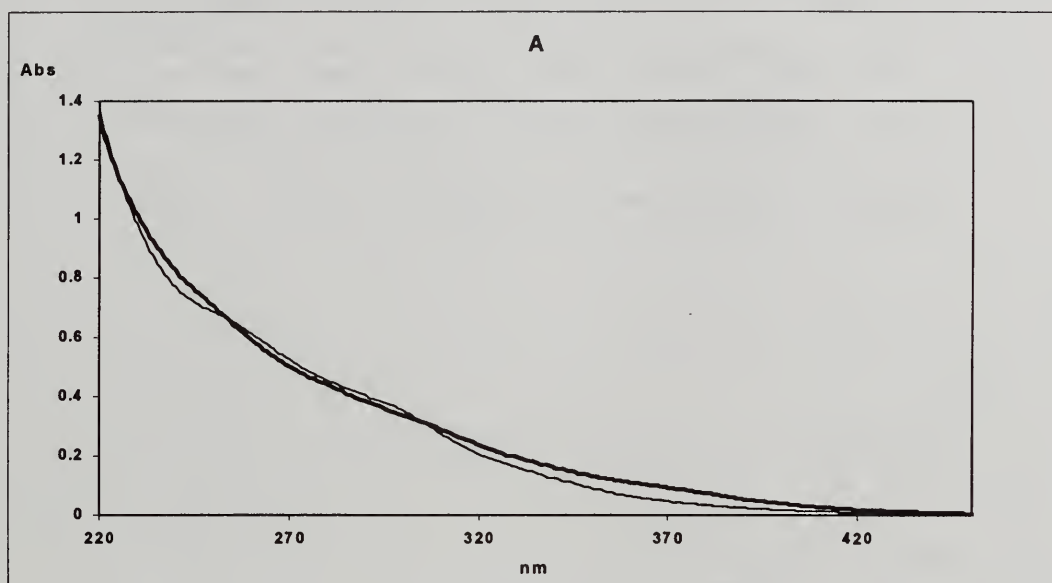


Figure 14 – Spectrum of Fraction A (— pH 9; — pH 3)

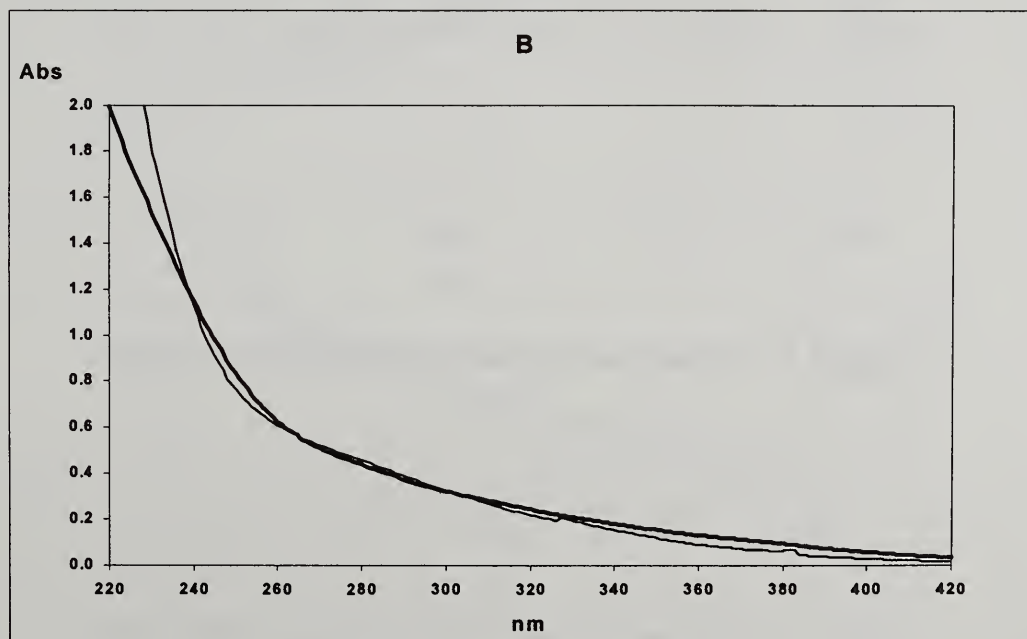


Figure 15 – Spectrum of Fraction B (— pH 9; — pH 3)

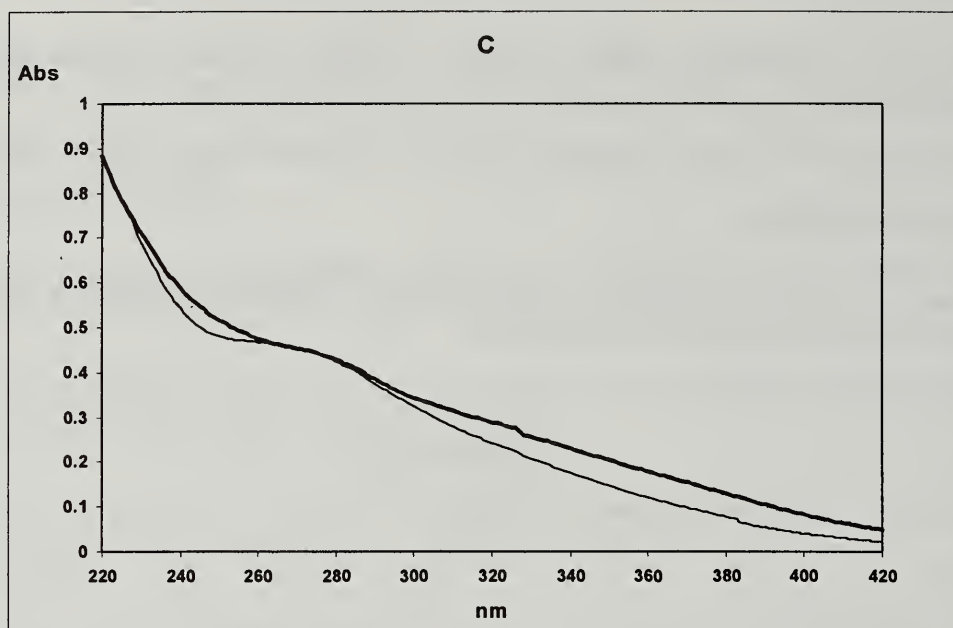


Figure 16 – Spectrum of Fraction C (— pH 9; — pH 3)

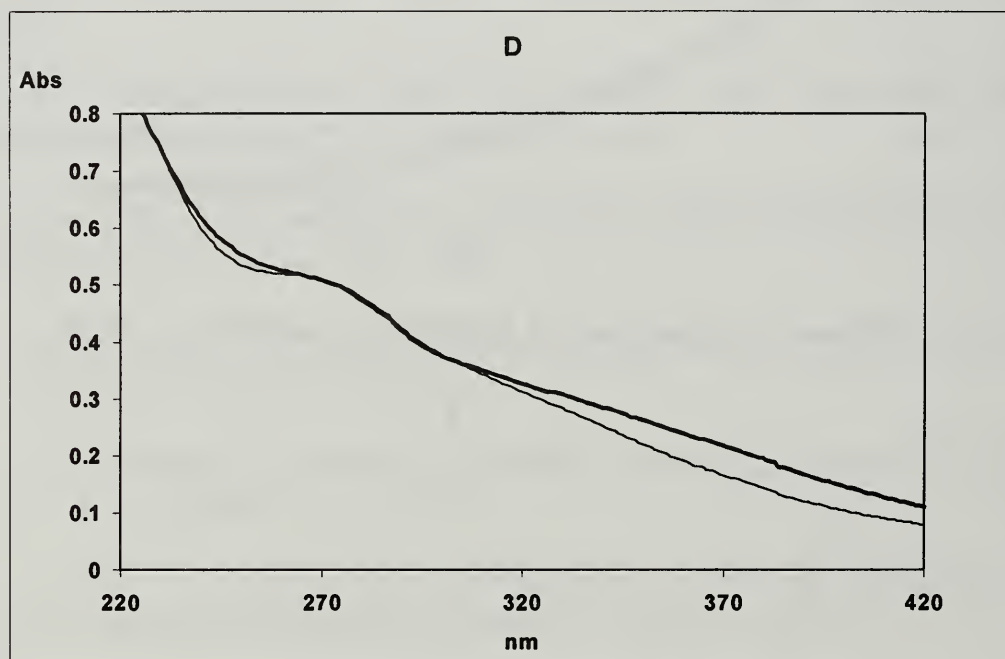


Figure 17 – Spectrum of Fraction D (— pH 9; — pH 3)

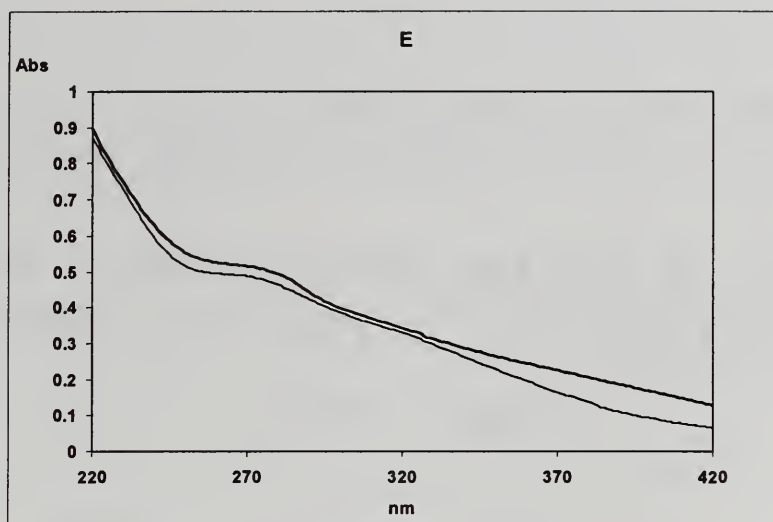


Figure 18 – Spectrum of Fraction E (— pH 9; — pH 3)

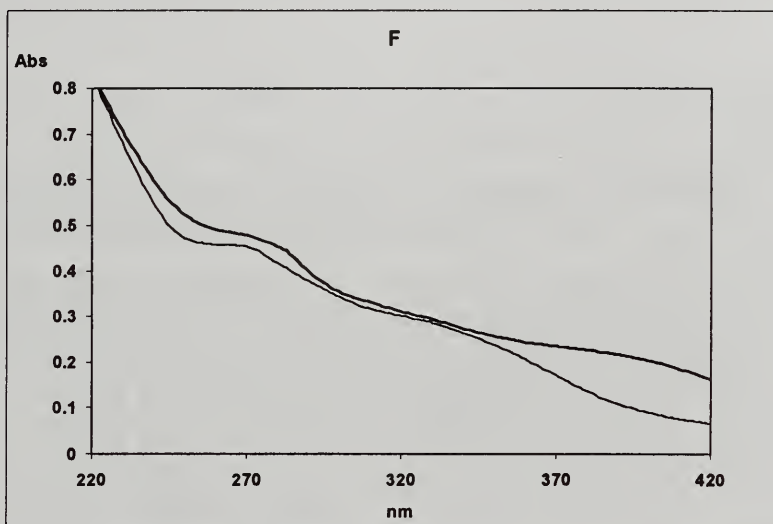


Figure 19 – Spectrum of Fraction F (— pH 9; — pH 3)

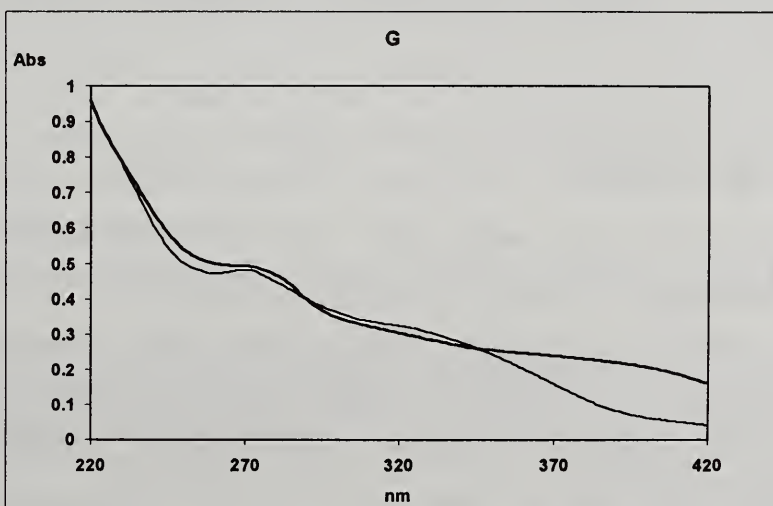


Figure 20 – Spectrum of Fraction G (— pH 9; — pH 3)

APPLICATION OF COLOR MEASUREMENT OF PROCESS INTERMEDIATES FOR PROCESS CONTROL

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INTRODUCTION

Since sugar cane is a natural raw material, the quality of juice is expected to vary from one region to another in the country. Variation in the nature and concentration of colorants are also likely to occur in different varieties of sugar cane. In a constant process, since the concentration of the colorants will have an impact on the color of sugar, and the present sugar business requires quality plantation sugar with low color, it is essential to monitor the concentration of colorants at every stage of the process in order to identify the stations where the color is being developed along the process, and to control the process so as to make the process effective. With this intention in mind, experiments were carried out to determine the concentration of colorants at various stages of process, when we process the juice extracted from different varieties, which are common in our command area of India. The colorant concentration was studied at different stages of processing, such as during the main season (November to June) and the special season (August to September).

MATERIALS & METHODS

Chemicals. Triethanolamine (AR Grade), HCL and NaOH (AR Grade) were used for the analysis of color. Solutions and dilutions were made in double distilled water.

Instruments. UV-visible spectrophotometer Shimadzu Model No. UV2401-PC wavelength accuracy 0.3 nm, bandwidth 2 nm. pH meter : Control Dynamics. Table refractometer: Schmidt & Haensch (ATR – SW). Millipore membrane filter assembly was used for filtration using membrane filter of 0.45 μ pore size.

Methods

Sugar samples were analyzed using TEA buffer according to ICUMSA Method GS2/3-9 and the intermediates samples were analyzed by diluting the samples with double distilled water according to ICUMSA Method GS 1-7.

Experimental

In order to study the concentration of colorants in different varieties and its impact on the color of sugar, composite samples of juice, process intermediates and sugar were collected and analyzed for color. Composite samples of juice, process intermediates and sugar were also collected continuously for different days during the main and special season and analyzed for color to determine process efficiency.

RESULTS AND DISCUSSION

The color of mixed juice varied between 16,000 and 35,000 during the special season and it varied between 48,000 and 77,000 during the main season (Tables 1, 2 and 3). This may be due to various factors such as maturity, post harvest deterioration, harvesting condition and also the quantity of tops and trash coming along with the cane.

The average color removal at the clarification stage during the special season was around 29% and the same was around 65% during the main season (Figures 1 and 2). The difference between the color of mixed juice (MJ) and clarified juice (CJ) reflects the efficiency of clarification. The difference in the color value of CJ and unsulfured syrup (US) of both the seasons shows that the evaporation was conducted rapidly, maintaining the critical parameters like temperature in control. During the syrup sulfitation process, a color reduction of 12% and 15% was observed during the special and main season, respectively.

The average color of white sugar in the main season was 168 ICUMSA units where the color of mixed juice was 62,287; the color of sugar in the special season was 124 ICUMSA units where the color of MJ was 27,555. In spite of 65% color removal at the clarification stage during the main season, the color of the sugar was higher than that of the special season where the MJ color was lower. This clearly indicates that the nature of the colorants in the mixed juice have an impact on the color of the sugar.

Figure 3 shows that, in an undisturbed process, when we process the juice with varied concentration of colorants, the color of sugar produced is directly proportional to the concentration of the colorants in the mixed juice. On the other hand, the average color value of sugar for different varieties was 153 units where the color of mixed juice was slightly higher than the average value of the main season (Table 3). Hence, even though the mixed juice color may be higher and if the color removal efficiency increases at various stages, the color of sugar can be reduced.

Hence it is clear that the nature and concentration of colorants present in cane plays a major role in the color of sugar produced irrespective of the efficiency of color reduction at various stages along the process. Further study has to be carried out to determine the nature of colorants and its concentration in different varieties. Also the possibilities of introducing new systems like syrup clarification to remove the colorants in order to produce of sugar of low color.

An attempt was made to correlate the color of mixed juice with the color of sugar. From Figure 5, it is evident that the color of sugar increases as the color of mixed juice increases in a consistent process.

CONCLUSION

The present scenario in the sugar industry shows that the color of sugar has become a very important parameter, which determines the marketability of plantation white sugar and also the price in the domestic and in the international market. Hence it is essential for the sugar industry to produce sugar of low color value by monitoring the process effectively. The results of our experiments show that the color values at different stages of process such as clarification, evaporation, syrup sulphitation, pan boiling and crystallization shall give an idea about the process efficiency and can identify the stations which deviate from ideal performance. It is also evident from the data that the color of mixed juice does influence the color of sugar. Based on the data obtained from our experiments, the following conclusions were made:

- Color analysis of process intermediates such as clear Juice, unsulfured syrup, sulfured syrup and massecuites will be highly useful for identifying the areas where color is being created in the process or when it is not being effectively removed.
- The color of sugar depends on the color of mixed juice when variations in process conditions are low.
- Color removal at the clarification and syrup sulphitation stage should be as high as 70–75% and 18-20% respectively to produce sugar of low color irrespective of the color of the mixed juice.
- Color formation at the evaporation stage should be minimized.

RECOMMENDATIONS

- The Spectrophotometer determination of color of process intermediates should be included as a process control parameter.
- Adoption of ICUMSA methods of analysis for all the parameters.
- Maintenance of optimum pH in the entire process of sugar manufacture.

- Maintenance of recommended temperature at every stage of sugar manufacture.
- Further studies to be carried out to determine the nature of colorants and its concentration in different varieties and the possibility of introducing new systems to remove the colorants effectively in order to obtain sugar of low color.

ACKNOWLEDGMENTS

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Table 1 : ICUMSA Color of Intermediates (Special season)

S.No	Mixed Juice	Clear Juice	Un Sul. Syrup	Sul. Syrup	Sugar
1	16,717	13,690	9,427	10,220	108
2	24,620	19,505	21,734	18,646	190
3	37,220	19,243	22,367	19,330	138
4	34,272	16,987	20,686	18,203	113
5	21,733	19,260	20,466	18,026	123
6	35,133	25,046	21,324	21,124	110
7	33,568	24,169	26,103	22,710	147
8	27,220	21,776	22,647	18,797	114
9	26,143	20,392	19,576	16,052	104
10	18,926	15,709	16,966	14,421	94
Average	27,555	19,578	20,130	17,753	124

Table 2 : ICUMSA Color of Intermediates (Main season)

S.No	Mixed Juice	Clear Juice	Un Sul. Syrup	Sul. Syrup	Sugar
1	48,820	22,194	24,350	22,191	195
2	54,520	21,808	19,627	18,253	135
3	65,480	24,882	23,140	21,289	162
4	77,888	25,373	27,770	19,814	211
5	66,458	19,568	22,475	16,923	137
6	60,557	21,820	18,384	17,410	173
Average	62,287	22,608	22,624	19,313	169

Table 3 : ICUMSA color of Intermediates of Different Varieties

Sample	MC 707	Co 86032	CoC 90063	Co Si 95071	Average
Mixed Juice	72,560	65,270	62,746	63,540	66,029
Clear Juice	23,373	21,116	23,560	22,970	22,755
Un Sul. Syrup	21,631	20,950	22,346	23,180	22,027
Sul. Syrup	20,213	15,483	16,574	18,490	17,690
Sugar	185	167	128	135	154

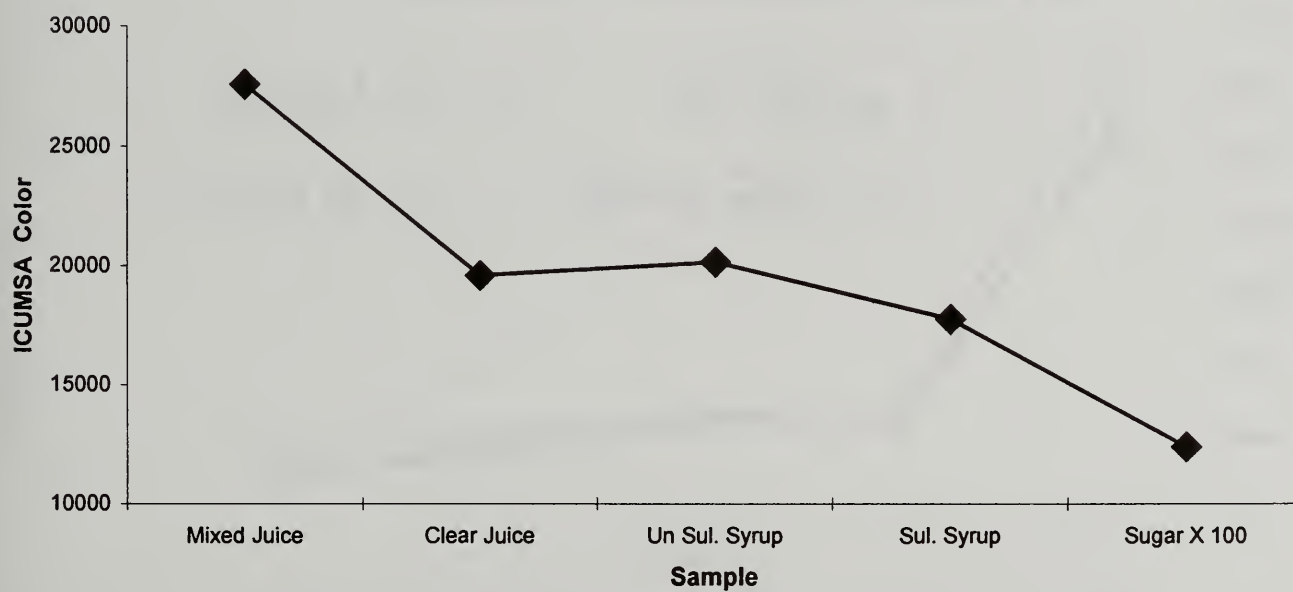
Fig 1 : Color of Intermediates (Special season)

Fig 2 : Color of Intermediates (Main season)

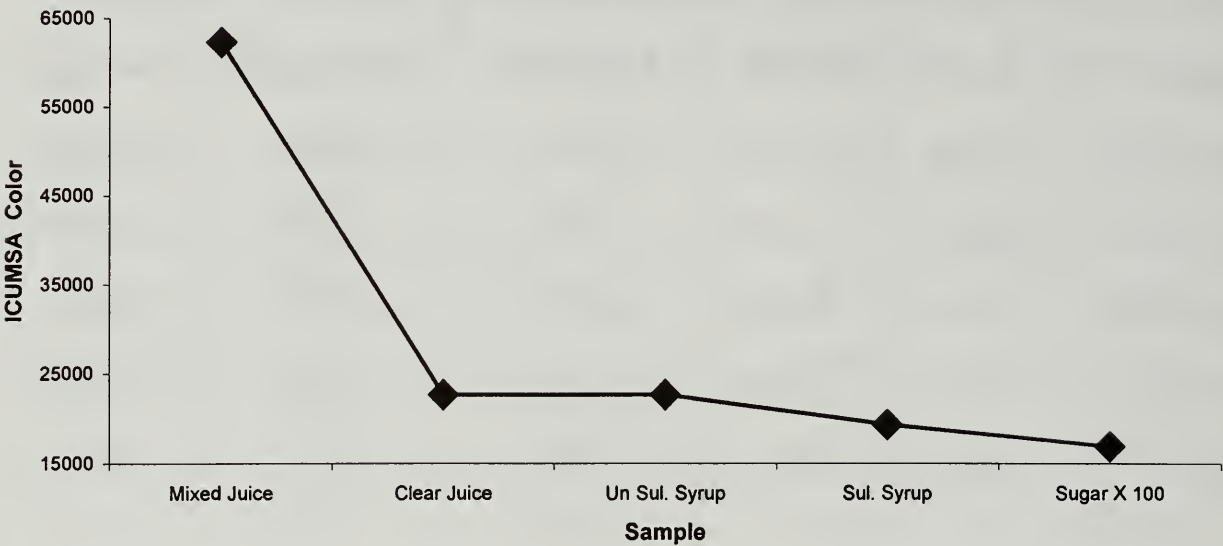


Fig 3 : Color of Intermediates of Different Varieties

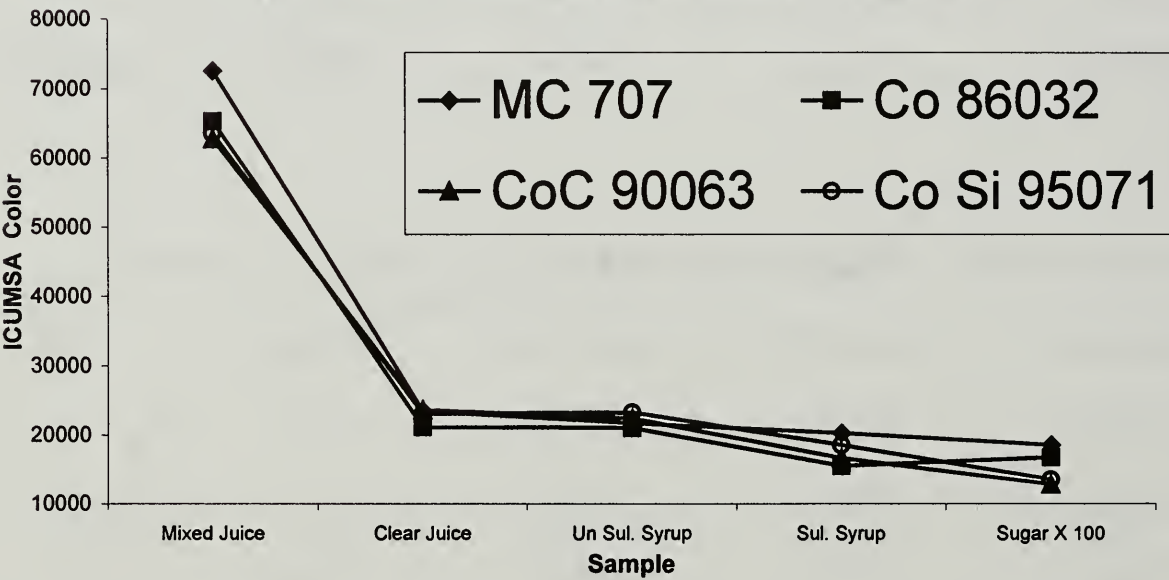


Fig 4 : Average color of Intermediates of Different Varieties

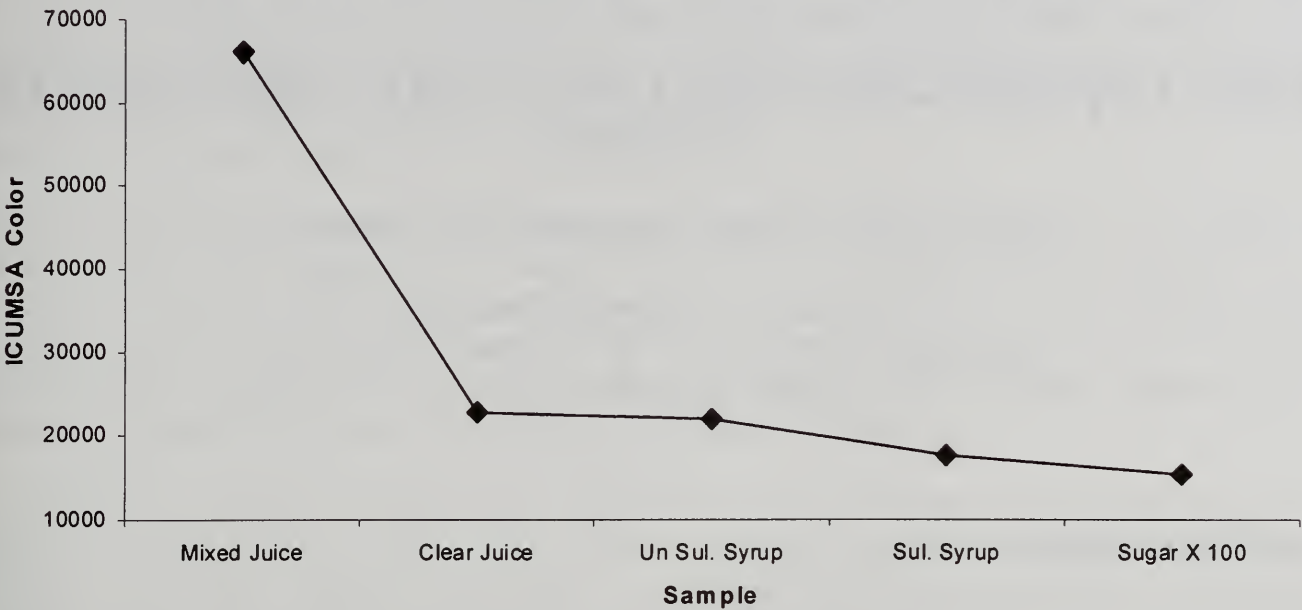
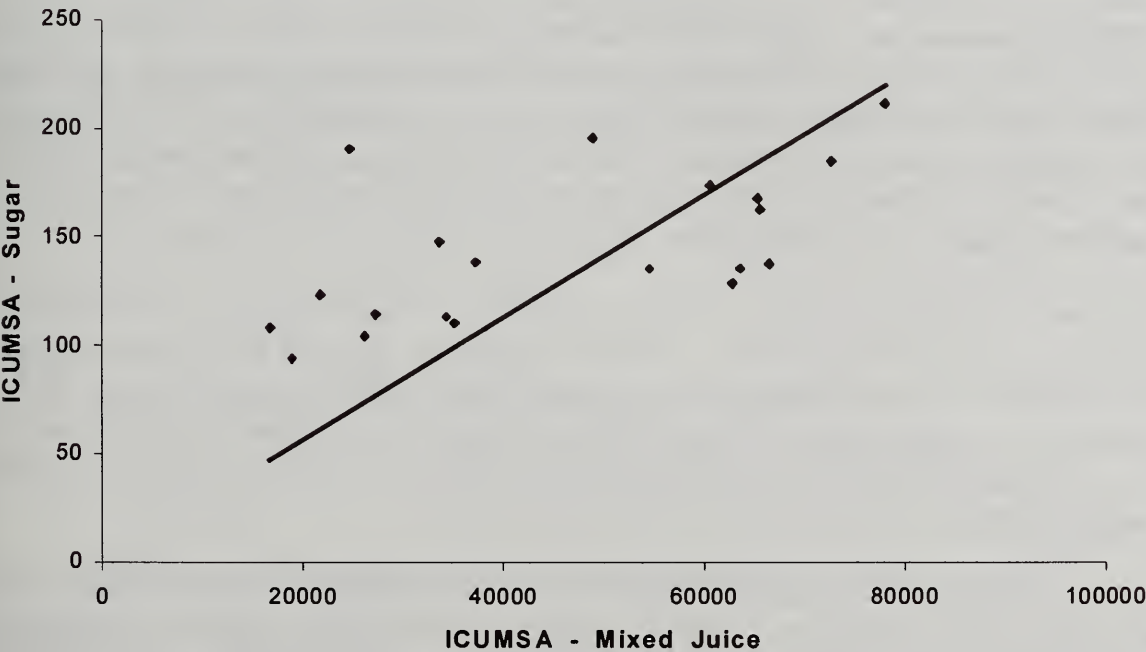


Fig 5 : Correlation between Mixed Juice Color and Sugar Color



IONIC LIQUIDS: GREEN SOLVENTS FOR CARBOHYDRATE STUDIES

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INTRODUCTION

Carbohydrates are compounds the U.S. sugar industry is well acquainted with. However, if the U.S. sugar industry wants to grow stronger in this new age then new uses for carbohydrates must be found. Increasingly, new technologies, new laws, and public awareness are ushering in plant matter as a new materials base for the new millennium. The use of plant matter as this new materials base is dubbed a “carbohydrate economy” by David Morris (<http://www.carbohydrateeconomy.org/>). Carbohydrates, the fundamental materials of plant matter, can be converted into chemicals, energy, textiles, building materials, paper, and many other industrial products, but a major barrier to utilization is the availability of cost effective, clean separation and processing technologies. The newly emerging field of ionic liquids as solvents offers a unique opportunity to investigate such new technologies from several perspectives having the same overall objectives.

The Center for Green Manufacturing has explored utilization of ionic liquids in biotechnology and the generation of renewable feedstocks. We are working to develop new separations and processing technologies using ionic liquids to better handle heterogeneous plant components and to develop advanced (bio)catalysts for monomeric and polymeric conversions. One such success has been the use of ionic liquids for dissolving the natural polymer cellulose. This work has led to patentable technology.

Cellulose can be dissolved in ionic liquids without first derivatizing and subsequently easily regenerated in a range of structural forms including fibers and membranes without requiring the use of harmful or volatile organic solvents.¹ By understanding the solublizing properties of ionic liquids, and how the properties may change within this class of solvents, a range of cellulose solvents are accessible, and can allow the construction of different macroscopic materials. Therefore, the need for fundamental physical measurements for carbohydrates in ionic liquids such as solubilities and partitioning data are necessary.

RENEWABLE RESOURCES AND IONIC LIQUIDS

A major principle in the Green Chemistry & Engineering discipline is that wherever technically and economically practical a raw material or feedstock should be renewable, rather than depleting.² New separations technologies to better handle heterogeneous plant components and advanced (bio)catalysts for monomeric and polymeric conversions are key goals and opportunities. In fact, Mantovani and Vaccari have pointed out that the idea of green manufacturing is gaining ground in the sugar industry.³

At the previous Conference on Sugar Processing Research in Porto, Portugal, we outlined our vision of how green chemistry can shape the future of the sugar processing industry.⁴ We concluded "there has been, and should continue to be...an increase in carbohydrate-based materials used in the chemical industry." We are working to form a collaboration between experts within academia, government, and the Sugar Industry to investigate sugarcane as a renewable resource.⁵ Sugarcane is more than just a source for sucrose.

Ionic liquids in catalytic reactions are a burgeoning area of research including biocatalysis.⁶ Reports in the literature have shown that effective enzymatic transformations can indeed occur within an ionic liquid matrix.⁷⁻⁹ In addition, ionic liquids have been successfully employed in extractive fermentations.^{10,11} A group from Japan have synthesized a new class of ether-containing ionic liquids capable of dissolving carbohydrates.¹² These results are exciting and suggest that a viable research program in the dissolution and chemical modification of plant-based materials is possible.

Ionic liquids are possessed of a number of properties that may be of importance in their application as extractive media in liquid-liquid extraction processes. They are liquid at room temperature, but in fact have an enormous liquid range of 300°C that is larger than that of water and offers the potential for considerable kinetic control over extractive processes. They are good solvents for a wide range of inorganic, organic and polymeric materials. Ionic liquids are known to be water insensitive and which are immiscible with water, thus enabling the concept of liquid-liquid extraction from aqueous media.¹³⁻¹⁵ Ionic liquids are relatively undemanding and

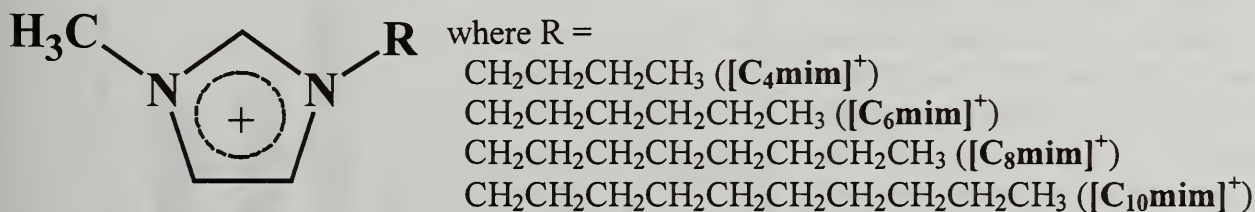


Figure 1. Structural variation in room temperature ionic liquids.

inexpensive to manufacture. A typical example of such an ionic liquid based on *n*-alkylmethylimidazolium cation is shown in Figure 1. The anion commonly used to make water immiscible ionic liquids is hexafluorophosphate but could also be tetrafluoroborate. The R-group of the cation may be selected also and could be chosen to be methyl, ethyl, etc giving interesting changes to the physical properties of the ionic liquid. They appear to have a capacity for organic molecules including various classes of aromatic compounds consistent with oxygenated aromatic compounds currently recovered from plant biomass.¹⁶

Ionic liquids offer a new class of solvents with environmentally favorable characteristics that are just being explored within the realm of biological materials and biomolecules. Therefore, the need for fundamental physical measurements such as solubilities and partitioning data are necessary. One class of biomolecules that we have chosen to study within ionic liquids is carbohydrates. Preliminary results in The University of Alabama's Center for Green Manufacturing laboratories have demonstrated that cellulose can be directly dissolved in $[C_4mim]Cl$.¹ Carbohydrates are indeed the predominant chemical compounds present in plant biomass.

Table 1 presents our results to date measuring solubilities of several key mono- and disaccharides in ionic liquids. $[C_6mim][PF_6]$ and $[C_8mim][PF_6]$ are hydrophobic ionic liquids. They are immiscible with water. The simple sugars investigated are insoluble in these hydrophobic ionic liquids. $[C_6mim][Cl]$ is a hydrophilic or water miscible ionic liquid. Simple sugars are indeed soluble in this ionic liquid. There appears to be a trend that suggests that solubility is dependent upon size of the carbohydrate. Sucrose, a disaccharide, was observed to have the lowest solubility in ionic liquids. The aldoses, glucose, a C6 sugar, and xylose, a C5 sugar, have similar solubilities in $[C_6mim][Cl]$. Fructose, a C6 sugar and a ketose sugar, had the greatest solubility observed.

Table 1. Solubility of sugars in ionic liquids at 22°C.

	$[C_6mim][Cl]$	$[C_6mim][PF_6]$	$[C_8mim][PF_6]$
Xylose	0.050 g/mL	Insoluble	Insoluble
Fructose	0.062 g/mL	Insoluble	Insoluble
Glucose	0.044 g/mL	Insoluble	Insoluble
Sucrose	0.005 g/mL	Insoluble	Insoluble

Fig. 2 presents our results from partitioning experiments of sucrose between two hydrophobic ionic liquids and water systems. Sucrose prefers the water phase in these two phase systems.

We are developing ionic liquids as solvents for dissolution and processing of natural carbohydrates and ligands. The work is aimed at developing efficient separations and processing media to reduce the cost and energy usage in utilization of renewable feedstocks (plant materials) to make new products. These results are exciting and taken together suggest that a viable research program in the dissolution and biological or chemical modification of plant-based materials is possible. Also, these preliminary results on solubility of sugars in ionic

liquids indicate that ionic liquids can be tailored to the ambient temperature dissolution of simple carbohydrates. Many derivatization and catabolic processes will be applicable in this environment and fractionation of products will be a key to success.

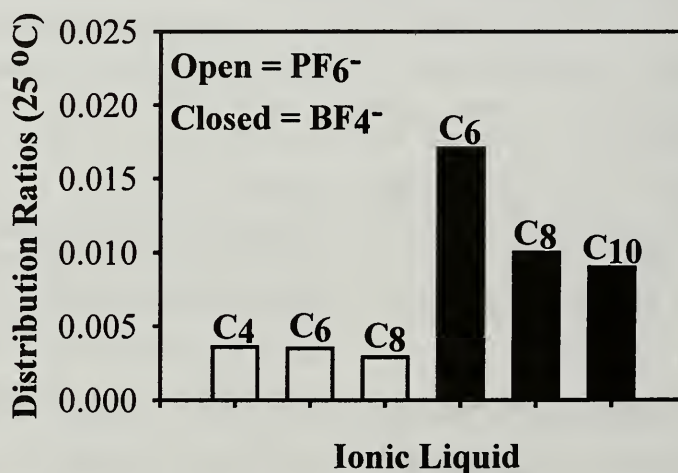


Figure 2. Sucrose distribution ratios in IL/water systems.

SUMMARY AND CONCLUSIONS

Plant/crop-based renewable resources are already being developed as alternatives to the fossil fuel-based materials currently relied upon by the chemical industry, and ionic liquids are beginning to play a role. The need for The Sugar Processing Industry to begin developing its renewable resources is now upon us. In the 21st century the sugar industry can provide not only a major food source, but also consumer goods and materials that are better for the environment and cost effective.

ACKNOWLEDGEMENTS

Our research efforts in Green Chemistry & Engineering are currently supported by the following agencies: Division of Chemical Sciences, Office of Basic Energy Sciences, Office of Energy Research, U. S. Department of Energy (Grant No. DE-FG02-96ER14673); U. S. Environmental Protection Agency (Grant No. R82825701-0); and the U. S. National Science Foundation (Grant No. EPS-9977239).

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CERAMIC MEMBRANES FOR CANE SUGAR JUICE CLARIFICATION

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ABSTRACT

Ceramic membranes are increasingly being used in the sugar industry. Thanks to their unique thermal, chemical, and mechanical properties, ceramic membranes offer several advantages over polymeric membranes, stainless steel membranes, and conventional filtration techniques (e.g. rotary drum filtration, decantation, centrifugation, and media filtration). Ceramic membrane technology can be successfully employed at various sugar processing stages including clarification of raw juice during raw sugar production and clarification of raw/single-effect juice, upstream to chromatography or ion-exchange step, during refined sugar production. The presentation will highlight the ability of ceramic membranes to remove suspended/colloidal solids, reduce color, and increase sugar purity, making them a cost-effective and preferred method of filtration.

INTRODUCTION

Ceramic membranes today run the gamut from "A" to "Z" in terms of materials (from alpha alumina to zirconia) and applications (from apple juice filtration to zinc oxide processing) and benefits (from adding value in production streams to meeting zero discharge requirements) -- but they are, relatively speaking, the new kids on the block in the world of liquid crossflow filtration. As such, new applications and benefits are constantly being found for the use of ceramic membranes. This paper will explore some of these opportunities in the cane sugar industry and highlight the unique characteristics of ceramic membranes.

Crossflow Filtration with Ceramic Membranes

Crossflow filtration is a continuous process, in which the feed stream flows parallel (tangential) to the membrane filtration surface and generates two outgoing streams. A small fraction of feed, called permeate or filtrate, separates out as purified liquid passing through the membrane. The

remaining fraction of feed, called retentate or concentrate, contains particles rejected by the membrane. The separation is driven by the pressure difference across the membrane, referred to as transmembrane pressure. The parallel flow of the feed stream, combined with the boundary layer turbulence created by the crossflow velocity, continually sweeps away particles and other material that would otherwise build up on the membrane surface. As a result, crossflow membrane filters inherently maintain high filtration rates, compared to those typically seen with conventional dead-end filters, where the feed flow is perpendicular to the filter surface.

The origin of Membralox[®] ceramic membranes can be traced to the EURODIF Uranium enrichment project in Europe nearly 30 years ago. These membranes were originally developed for gas-gas separation. The first microfiltration (MF) ceramic membranes for liquid separation were introduced in the early eighties. This was followed by subsequent introduction of ultrafiltration (UF) ceramic membranes in the late eighties and more recently with the launch of "loose" nanofiltration (NF) ceramic membranes.

Ceramic Membrane Elements and Modules

Ceramic membranes are constructed from multiple ceramic layers and formed into an asymmetric, multichannel element. The ceramic membranes under discussion in this paper are manufactured using alumina, zirconia or titania depending on the desired pore size of the membrane and then sintered onto an alpha alumina support. MF membranes with pore size 0.1 μm and higher are made of pure alpha alumina. UF membranes, with pore size between 20 nm and 100 nm, and NF membranes, with pore size of 1000 and 5000 Dalton MWCO, are made of zirconia and titania, respectively. Several membrane pore sizes are available to suit specific filtration needs - in MF/UF/NF ranges - from 5 microns down to 1000 Daltons (MWCO). Tables 1 and 2 give the characteristics of the ceramic membranes.

Table 1: Ceramic MF/UF/NF Membranes

Membrane Layer	Pore Size
α -Alumina	0.1, 0.2, 0.5, 0.8, 1.4, 2.0, 5.0 μm
Zirconia	20, 50 and 100 nm
Titania	1000 and 5000 Dalton (MWCO*)

*MWCO - Molecular Weight Cut-off

Ceramic membrane elements are available in different shapes (circular, hexagonal, square, etc.) and various feed channel diameters. The membrane elements under discussion are hexagonal in shape and are available in three different feed channel diameters – 3, 4, and 6 mm ID – providing a nominal membrane filtration area from 0.24 to 0.36 m^2 . Each element is about 1 m long and has 19 or 37 parallel feed channels within the ceramic support. The multichannel construction of the membrane element provides a higher membrane packing density than a tubular element of the same length. Figure 1 illustrates the available membrane geometries.

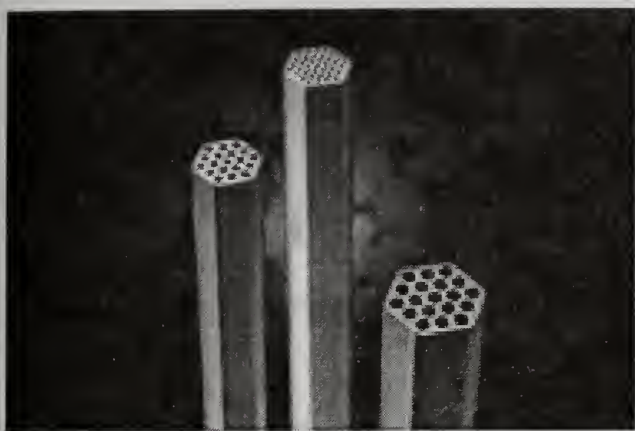


Figure 1.

Table 2: Clean Water Permeability ($\pm 15\%$) at 20°C

Membrane Pore Size	Permeability (l/hr.m ² .bar)
Microfiltration	
0.1 μm	1500
0.2 μm	2000
0.5 μm	4500
0.8 μm	7500
1.4 μm	11000
5.0 μm	23000
Ultrafiltration	
20 nm	300
50 nm	900
100 nm	1800
Nanofiltration	
1000 Dalton (MWCO)	30
5000 Dalton (MWCO)	60

The gasketed membrane elements are assembled within housings, available in 316L SS (standard), PVDF, or other alloys. The fully assembled housing (see Figure 2), called a membrane module, can have a filtration area between 0.24 m² (2.6 ft²) and 21 m² (226 ft²). A typical industrial installation will have a several of these modules arranged in series and/or parallel configuration. Table 3 lists the various membrane modules and the corresponding filtration areas. The membrane modules can withstand elevated temperatures (up to 300°C), extremes of pH (0 to 14), high operating pressures (up to 150 psi) without any concern for membrane compaction, delamination or swelling. This makes ceramic membranes suitable for many applications where polymeric and other inorganic membranes cannot be used. Additionally, ceramic membranes are ideal for in-place chemical cleaning at high temperatures, while using caustic, chlorine, hydrogen peroxide, ozone and strong inorganic acids, etc. Steam sterilization is also possible. Finally, these membranes can also be backpulsed, which is a permeate flow reversal technique to reduce membrane fouling and increase filtration efficiency.



Figure 2.

Table 3: Membrane Modules

Module	Number of membrane elements per module	Membrane filtration area per module (m ²)
<i>4 mm ID channel size</i>		
1P19-40GL	1	0.24
3P19-40GL	3	0.72
7P19-40GL	7	1.7
19P19-40GL	19	4.6
37P19-40GL	37	8.9
60P19-40GL	60	14.4
<i>6 mm ID channel size</i>		
1P19-60GL	1	0.36
3P19-60GL	3	1.08
12P19-60GL	12	4.3
22P19-60GL	22	7.9
<i>3 mm ID channel size</i>		
1P37-30GL	1	0.35
3P37-30GL	3	1.05
7P37-30GL	7	2.4
19P37-30GL	19	6.6
37P37-30GL	37	13
60P37-30GL	60	21

Industrial Applications

Ceramic membrane filtration provides a particularly attractive alternative in the cane sugar industry, replacing such conventional treatments as holding/decanting, diatomaceous earth (DE) and cake filtration, rotary drum filtration and centrifugation. Membranes produce superior clarity juice and deliver higher yields compared to conventional clarification processes.

In the filtration of sugar cane juice, ceramic membranes can be used in several different stages in the raw and refined sugar production. One interesting opportunity is in the MF/UF of clarified juice (7 – 14 Brix) and/or pre-evaporated juice (20 – 25 Brix) as a pretreatment prior to ion exchange or chromatographic separations. Pretreated and filtered juice is softened, evaporated and purified using ion exchange and chromatographic processes leading to a better quality refined sugar. Typical operating conditions include feed temperatures of 90 – 100°C, high crossflow velocity (4 – 7 m/s) and transmembrane pressures up to 5 bar. The need for filter aid purchase and disposal is eliminated. In some applications, the cost of equipment necessary to dewater filter-aid sludge may be comparable to the cost of the membrane system. Table 4 lists the process parameters in cane sugar juice clarification.

Table 4: Process Parameters for Cane Sugar Juice Clarification

Ceramic membrane pore size	0.05 – 0.2 micron
Temperature	90 – 100°C
Crossflow velocity	4 – 7 m/s
Average flux	100 – 300 LMH
Recovery	90 – 98%
Turbidity removal	97 – 99%
Sugar purity increase	1 – 2%
Color reduction	2 – 10%

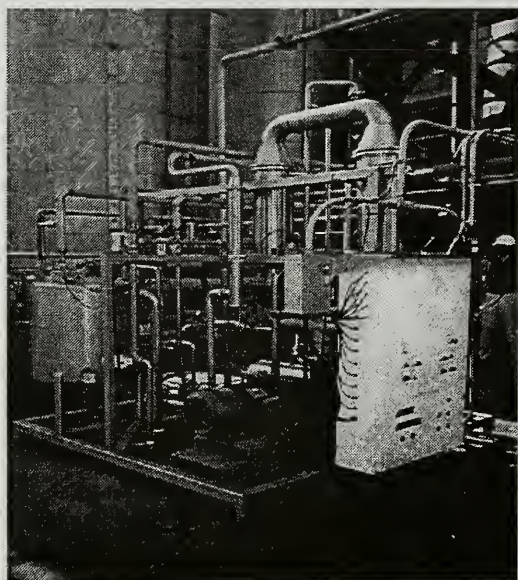
Another application for ceramic membranes in the cane sugar industry is the recovery and reuse of spent caustic solution. Hot caustic solutions are used to clean a variety of equipment such as tanks, mixers, evaporators and crystallizers. This is necessary to maintain proper sanitary conditions between batches. The spent caustic solutions contain suspended solids (pulp, low molecular weight proteins, organic polymers, oligosaccharides, etc.) and additives such as wetting agents and surfactants. Typical operating conditions include feed temperatures of 50 – 70°C, high crossflow velocity (about 5 m/s) and transmembrane pressures up to 5 bar. Ceramic membranes are able to retain the suspended solids and allow permeation of the additives. Table 5 lists the process parameters in caustic recovery application.

Table 5: Process Parameters for Caustic Recovery

Ceramic membrane pore size	0.05 – 0.2 micron
Temperature	50 – 70°C
Crossflow velocity	5 m/s
Average flux	50 – 100 GFD
Recovery	85 – 95%
Turbidity removal	97 – 99%
Additive recovery	> 95%

A Membralox® ceramic membrane caustic recovery system was installed in 199 at Southern Gardens Citrus, Clewiston, FL (see Figure 3). The following advantages were reported in a recent paper (1):

- Reduced annual caustic consumption by 30%
- Reduced annual operating costs by \$80,000
- Reduced annual energy consumption and waste disposal costs by \$10,000
- Payback period less than 1.5 years
- Meets environmental requirements

**Figure 3.**

CONCLUSION

Ceramic membranes are available in a wide range of membrane pore sizes, in multiple geometries allowing scale up from small to large process streams. Ceramic membranes deliver the following advantages in the cane sugar industry:

1. In cane sugar juice filtration
 - Long membrane life under the most demanding operating conditions
 - >97% turbidity removal improves performance of ion-exchange / chromatography systems
 - Reduced downstream processing costs
 - Increased sugar recovery and purity
2. In spent caustic recovery
 - Reduced caustic consumption
 - Reduced operating and waste disposal costs
 - Attractive payback time
 -

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FURTHER INSIGHTS ON THE FACTORY PERFORMANCE OF COLD, INTERMEDIATE, AND HOT LIME CLARIFICATION SYSTEMS

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ABSTRACT

Since 1996, Cora Texas factory in Louisiana has been operating intermediate lime clarification and was, therefore, one of the few U.S. factories that did not operate cold lime clarification. In an attempt to further improve clarification performance, the factory made the decision to convert to hot lime clarification during the 2000 grinding season. A comparative investigation of hot versus intermediate and cold lime clarification was undertaken to quantify performance. In cold liming, mixed juice (MJ) was incubated (8min) and then limed in a lime tank (4min), both at ~105° F. For intermediate liming, 50% of the MJ was heated (180-200° F) before incubation (8min), then limed in a lime tank (4min) at ~150° F. Hot liming was configured very similar to intermediate liming except that incubation time was increased from 8 to 12 mins, and that lime was added immediately after flash-heating (215°F; 30sec). Hourly samples across each of the three processes were collected over a six hour sampling period, on three consecutive days respectively, and these were repeated three times across the 2000 grinding season. For most clarification parameters investigated, both hot and intermediate liming performed much better than cold liming, and hot liming generally offered some extra advantages over intermediate liming. Less sucrose was lost to inversion reactions across both hot (season av. 0.79%) and intermediate (0.97%) lime processes than across cold liming (1.48%). By operating hot liming, the reduction in sucrose losses alone saved the factory approximately US\$283,000 over cold liming. Increasing the factory target pH of the final evaporator syrup (FES) from ~pH 6.0 to 6.3, in sampling period 3 in both hot and intermediate liming, caused a very marked reduction in sucrose inversion losses, particularly across the clarifiers and evaporators.

Dramatically less lime had to be added in hot liming compared to either cold or intermediate liming, with the factory consuming, on season average, only 1.01 lbs lime/ton cane compared to 1.28 for the 1999 grinding season when intermediate rather than hot liming was operated. Pre-heating 50% of the MJ in both intermediate and hot liming consistently removed color, dextran, and starch, but silicate levels were not significantly changed. Although, the fastest settling occurred in intermediate liming, ~ 2.1% (season av.) more turbidity removal (MJ to clarified juice [CJ]) occurred in both hot and intermediate liming compared to cold liming, with better CJ turbidity control. Subsequent FES and raw sugar turbidity values were better in hot liming. Markedly less color (~2.5%) formed on hot liming because of reduced retention time of liming, compared to ~17% color formation in cold and intermediate lime clarification. Dextran removal was best across hot liming and, as expected, dextran formed in the cold lime tanks. Using hot liming across the season, the factory observed 12-15% more heating capacity in the limed juice heat exchangers and a 90% reduction in the quantity of chemicals needed to clean the heat exchangers.

INTRODUCTION

It is well known that the degree of clarification has a great impact on boiling house operations, sugar yield, and refining quality of raw sugar. Several lime-clarification systems have been developed over the years including cold, hot, intermediate, fractional, and saccharate liming. Moreover, variations also occur within a particular clarification system, from factory to factory. Although many other parts of the world have changed from cold liming, mostly to hot liming, cold liming is still usually operated in the U.S. The main advantages of cold liming over other liming processes have been considered to be its simplicity of operation and less sucrose inversion (Chen, 1993), but these conclusions were drawn mainly from laboratory studies, which do not always reflect the complexity of factory processing streams which can change in seconds, and give no or little information on process control which is essential for engineers. Recent factory studies (Eggleston et al, 1999, 2002, and Eggleston, 2000a,b) have unequivocally shown that excessive inversion occurs in cold liming clarifiers, excessive color is formed on liming, pH and turbidity control are erratic, and turbidity removal is not adequate. Furthermore, with the introduction of mechanical harvesting of green and burnt billeted sugar cane in the 1990s in the U.S., especially in Louisiana, there has been an unfortunate large increase of impurities that require factory processing. Therefore, there is currently an even greater need to remove these extra impurities during clarification by using more advanced clarification systems than cold liming.

Although Eggleston (2000a,b) previously compared the performances of hot and cold lime clarification systems at two Louisiana factories across the 1998 grinding season, and compared intermediate and cold lime processes at a third Louisiana factory across the 1999 season (Eggleston et al, 2002), a systematic factory comparison of all three processes has not been reported. Such a study would be useful to help processors decide which clarification process is best to utilize; consequently, this study was undertaken to compare the performance of cold, intermediate, and hot lime clarification processes in a factory processing mostly billeted cane. As numerous potential benefits of intermediate liming over cold liming had been observed in a previous grinding season study at the factory in this study, just by preheating only 30% of the mixed juice before incubation and lime addition, for this study the factory increased the amount pre-heated to 50%. Also, unlike in previous factory studies, in this study raw sugar samples were analyzed to assess the impact of the clarification process on the raw sugar quality.

EXPERIMENTAL

This study was performed at Cora Texas raw sugar factory, Louisiana, USA, across the 2000 grinding season. The season average cane grinding rate and flowput were 521 short tons/h and ~1900 gallons/min, respectively, and ~99% of the cane processed was billeted, of which ~75% were green billets. All mixed juice (MJ) was pre-screened.

Factory Clarification Equipment and Procedures. The flow diagram for hot lime clarification is illustrated in Figure 1a. To convert to hot lime clarification the factory had to install new equipment, including a lime injector 4 inches below the bottom of the flash tank (Figure 2), a static mixer, and new pH measurement instrumentation to measure the pH of the flash heated limed juices at high temperatures. Factory measurement of pH was done by taking a continuous sample of the flash-heated limed juice and passing it through a cooling column before the pH measurement was made with a Van LondonTM industrial electrode.

Fig. 1a. Flow diagram of the hot lime clarification process

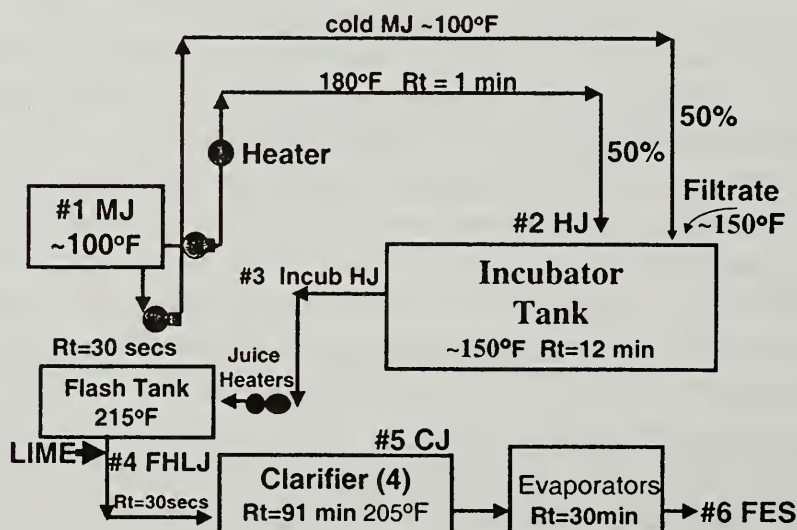


Fig. 1b. Flow diagram of the intermediate lime clarification process

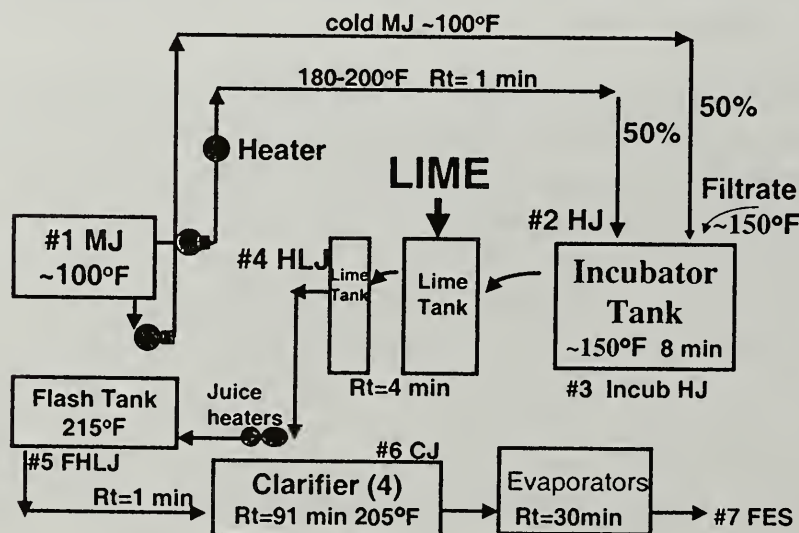


Fig. 1c. Flow diagram of the cold lime clarification process

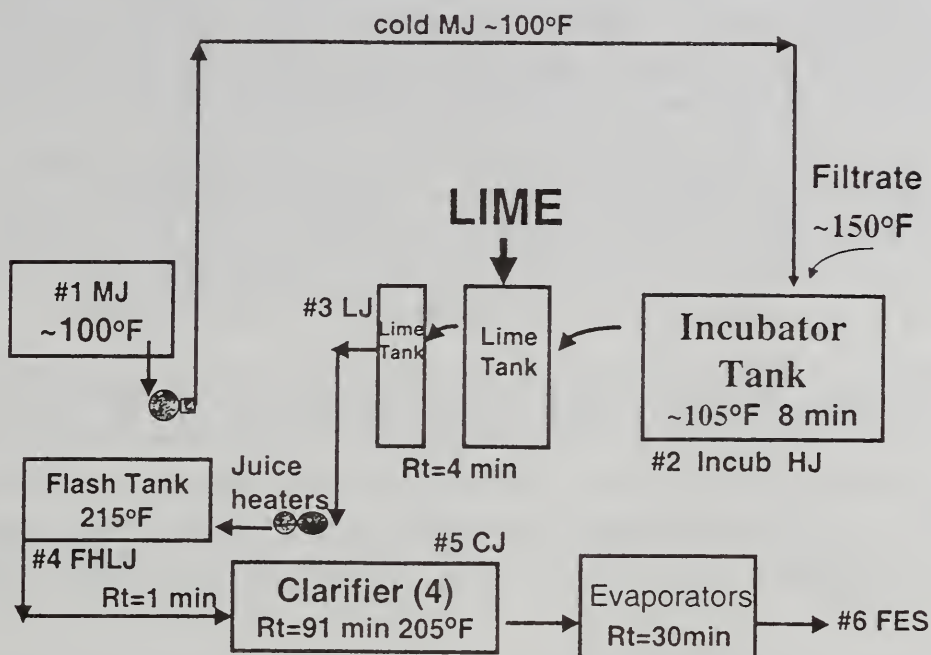
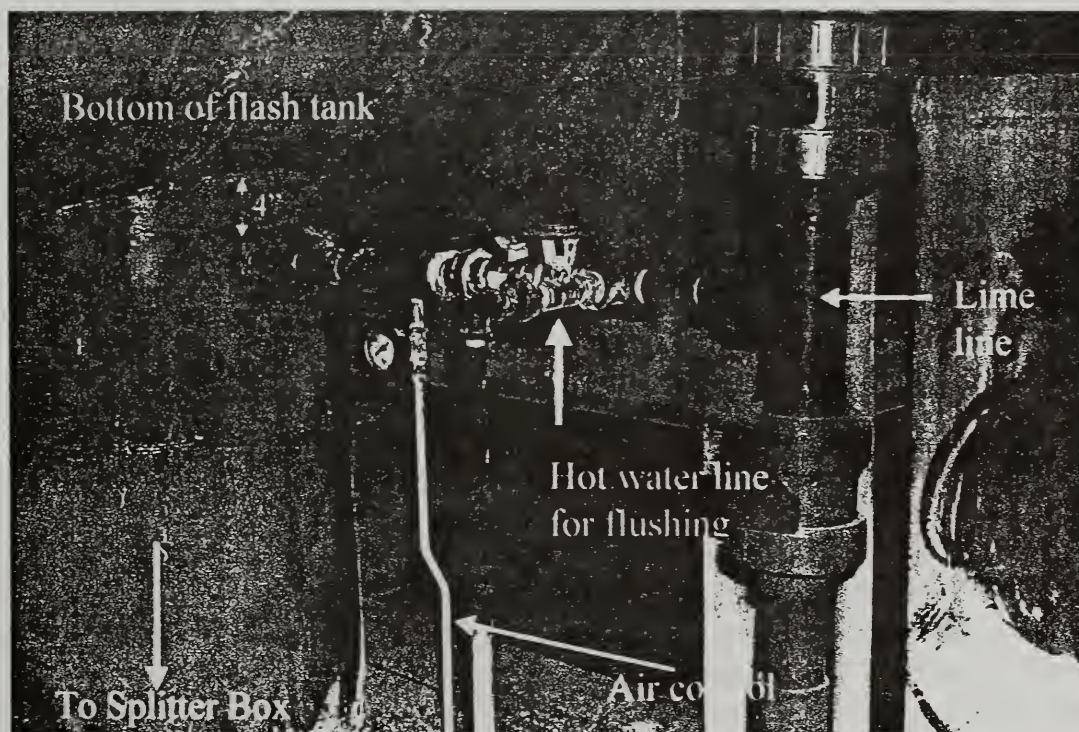


Fig. 2. Diagram of lime injection equipment for hot lime clarification



In hot liming (Figure 1a), 50% of the MJ was pumped to heaters and heated to 190-200° F before entering a juice incubation tank operated at ambient temperature (retention time 12min). The remaining 50% of “non-preheated or cold” MJ was pumped directly into the incubator tank. Filtrate from the clarifier mud filters, was also added in the incubation tank. The incubated juice was then flash heated to ~218-220° F to maintain constant temperature and remove air bubbles. Lime (~ 12 baume) was injected automatically into the flash heated juice 4 inches below the flash heater, mixed and then distributed into one of four clarifiers (see Figure 2). Polyelectrolyte flocculants were added (4ppm on clarified juice) before entering the clarifiers. In this study clarified juice was taken from the #4 Dorr Oliver 444 clarifier. The evaporation station consisted of two pre-evaporators and three triple-effect Robert’s type Calandria evaporators. Commercial α -amylase (2.5lbs/500 tons of cane) was added in the last bodies of the triple effect evaporators (max. temp 150 ° F).

In case of difficulties in hot lime clarification or cane quality problems, the factory still wanted the ability to revert to intermediate and cold liming when necessary, which was convenient for this study. Flow diagrams of the factory intermediate and cold lime clarification processes are shown in Figures 1b and c, respectively. For the conversion to intermediate liming from hot liming, two of the three tanks comprising the collective incubation tank were used as lime tanks ($R_t = 4\text{min}$) and pH measurement occurred there.

Cold liming was the same as intermediate liming, except that the recirculation pump after the mixed juice tank was stopped so that all the MJ entered the incubation tank without first being pre-heated (see Figure 1c). For all three clarification processes, the target pH of the final evaporator syrup was usually 6.0.

General Sampling. Because stored cane at the factory deteriorates more rapidly overnight, samples across cold, intermediate, and hot liming were taken between 8am - 5pm, on three consecutive days, respectively. The factory converted to cold or intermediate liming at least 1h prior to sampling to flush out the hot lime juice streams. Juices and syrups were carefully collected to prevent further chemical degradation reactions and/or microbial growth. Each sample was first collected in a large (250ml) container, and then ~25ml was poured into a 50ml container. Sodium azide (0.02%) was added to the 50ml container before putting in dry ice. Glucose, fructose, and sucrose concentrations were measured in juice from the small containers, usually the next day. Juice in the large containers was immediately cooled on ice, and Brix and pH were then measured at the factory. Sodium azide was then added and the juice stored on dry ice until transportation to, and storage in, a -80° C (or -112° F) laboratory freezer, subsequent to laboratory analyses. Flow rates in any factory fluctuate constantly, therefore, hourly samples were taken across a sampling period and grinding season to obtain precise averages. A six hour sampling period across each clarification system was repeated three times across the grinding season, in order to cover cane variety, environmental, and process parameter variations. The three clarification sampling period dates were: sampling period one, cold 3 Oct and Intermediate 4 Oct; sampling period two: cold 8 Nov, Intermediate 9 Nov and Hot 10 Nov; sampling period three: cold 12 Dec, Intermediate 13 Dec and Hot 14 Dec.

Hot Lime Sampling. Mixed juice (MJ), heated juice (HJ), incubated juice (incub J), flocculated heated limed juice (FHLJ), clarified juice (CJ), and final evaporator syrup (FES) were collected hourly over a six hour period (see Figure 1a). Retention times in the pipes and tanks were taken into account. Consequently, there was a 1min delay between sampling MJ and HJ, a 12min delay between

HJ and incub J, a 30sec delay between incub J and FHLJ, a 91 min delay between sampling FHLJ and CJ (residence time in the clarifier was calculated using tank dimensions and average flow rate), and a further 30 min delay between sampling CJ and FES, which was only an approximation. Because the factory had mechanical problems with the new hot liming pH system at the beginning of the grinding season, no valid hot liming samples were taken for the first sampling period.

Intermediate Lime Sampling. Sample collection was the same as for hot liming except that the three incubator tanks in hot liming were converted to one incubator tank ($R_t = 8\text{min}$) followed by two lime tanks (combined $R_t = 4\text{min}$), which is illustrated in Figure 1b. Consequently, a sample of heated limed juice (HLJ) was taken after incubation (Figure 1b).

Cold Lime Sampling. Sample collection was the same as for intermediate liming, except there was no heated juice (HJ) sample as cold mixed juice was pumped directly into the incubator tank (Figure 2c).

Raw Sugar Sampling. Raw sugar samples from each clarification process were also collected, at random, ~3h after sampling began, and stored in a desiccator before analyses.

Sucrose, Glucose and Fructose Concentrations. The determination of sucrose, fructose and glucose in cane juices and syrups by GC was based on ICUMSA method GS7/4-22 (1998) with modifications by Eggleston et al (2002).

Calculation of Sucrose Losses. % sucrose losses were calculated using the following formula of Schaffler et al (1985):

$$\% \text{ Sucrose lost} = \frac{\left(\frac{(\% \text{ Glu})_{\text{out}}}{\text{Brix}} - \frac{(\% \text{ Glu})_{\text{in}}}{\text{Brix}} \right) \cdot \text{MW}_{\text{SUC}} \times 100}{\frac{(\% \text{ Suc})_{\text{in}}}{\text{Brix}} \times \text{MW}_{\text{Glu}}}$$

where: MW = molecular weight, Suc = sucrose, Glu = glucose

Settling Rates and Mud Volumes of Flocculated Heated Limed Juices. See Eggleston et al (2002) for general settling and mud volume measurements and calculations. The flash-heated limed juice with flocculant added was brought to a boil before settling measurements were undertaken, in order to remove interfering gas bubbles.

Brix. The mean Brix of triplicate samples was measured using an Index Instrument temperature controlled Refractometer accurate to $\pm 0.01\text{Brix}$.

pH was measured at room temperature ($\sim 25^{\circ}\text{C}$ or 77°F), using an IngoldTM combination pH electrode calibrated at room temperature using two different pH buffers (pH 7 and 10). The electrode was connected to a Metrohm 716 DMS pH meter.

Color and Turbidity. These were measured as the absorbance at 420nm and calculated according to the official ICUMSA method GS2/3-9 (1994). Samples (5g) were diluted in triethanolamine /hydrochloric acid buffer (pH 7) and filtered through a 0.45 mm filter.

Dextran concentrations for duplicate composite samples (10g of each hourly samples were combined) were measured using the ASI-II (Sarkar and Day, 1986) method.

Starch concentrations for duplicate composite samples were measured using a colorimetric method (Godshall et al, 1990), based on the starch-iodine complex.

Calcium of composite samples as CaO was measured by EDTA titration following ICUMSA method GS8/2/3/4-9 (1994).

Silicate of composite samples as Si was measured by atomic absorption spectrometry.

Analysis of Data. Data were analyzed using PC-SAS 8.1 (SAS Institute, NC) software. Process (intermediate, cold and hot liming) and sample type were considered as fixed effects. Means comparisons were undertaken using Duncan's New Multiple Range Test.

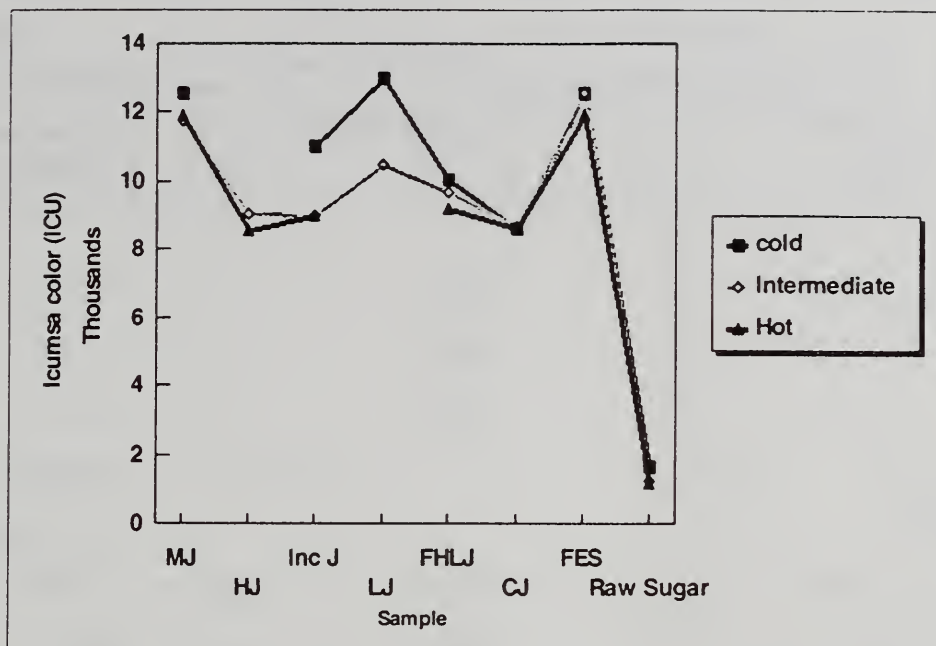
RESULTS AND DISCUSSION

Effect of the Three Different Lime Clarification Systems on Color Removal and Formation

For all three clarification systems, the color of the incoming mixed juice (MJ) differed little at the beginning and middle of the season, but decreased slightly at the end of the season. As illustrated in **Figure 3**, for hot and intermediate liming, pre-heating 50% of the mixed juice (HJ) before incubation caused marked color (season av. $\sim 26\%$) removal, which agrees with previous studies (Muller, 1921, Eggleston, 2000a and Eggleston et al, 2002). Furthermore, color removal in the HJ caused the incub J color to be lower than in cold liming (Figure 3). This heat induced color removal is considered to be associated with the precipitation of macromolecules, including polysaccharides (starch and dextran) and proteins (Armas et al, 1999). Color was also generally removed on incubation (Figure 3) in all three processes, which is most likely because of precipitation with lime salts in the added filtrate juice.

It is well known that color is formed on liming because of the alkaline degradation of invert, a reaction that is relatively fast, and increases with temperature and retention time. Because there was a 4 min retention time of liming in tanks for both cold and intermediate liming, $\sim 16\%$ color formation occurred. In hot liming, lime was added directly into the juice pipe just after flash heating (see Figure 2) and mixed with static mixers. This had the effect of reducing color formation to a range of only 1.7-2.8% across the season. However, in a previous study of hot liming at another Louisiana factory by Eggleston (2000a), no measurable color was formed.

Fig. 3. Color removal and formation



As expected, for all three processes color was removed by the settling process in the clarified juice (CJ) samples, and was formed across the evaporators because of the further reactions of sucrose inversion products. Although there were very little differences in CJ color for all three processes, final evaporation syrup (FES) and raw sugar colors were markedly lower in hot liming, and color control was better too (Figure 3).

Lime Consumption

Lime consumption was dramatically less in hot liming than both cold and intermediate liming, and is one of the major advantages of operating hot lime clarification. Lime addition was measured indirectly as the difference between the calcium concentrations in the mixed juice samples and in the samples where lime was added in the factory (Table 1). Much less lime had to be added in hot liming compared to either cold or intermediate liming. This dramatic difference was also reflected in the factory season average data. With the new operation of hot lime clarification across the 2000 grinding season, the factory consumed only 1.01 lbs lime/ton cane compared to 1.28 lbs lime/ton cane it consumed for the 1999 grinding season when intermediate liming was operated. Furthermore, even across the next grinding season in 2001 when hot liming was still operated but the factory target FES pH was increased to pH 6.3 from 6.0, still only 1.05 lbs lime/ton cane was consumed. It must also be noted that in sampling period 1, for both cold and intermediate liming (no data was available for hot liming), lime addition was much higher (Table 1). This is not really surprising because at the beginning of the grinding season, the pH of the extracted juice was unusually low (sometimes less than pH 4.5) which caused the factory to add caustic soda and lime in the cane wash water, but also necessitated the addition of more lime at the clarification stage.

Table 1. Calcium concentrations in composite samples.

Clarification Process / Sampling Period	Ca as CaO (ppm/Brix basis)		
	MJ	LJ/HLJ/FHLJ	Change MJ to LJ/HLJ/FHLJ ^a
<u>COLD</u>			
1	21.47	53.37	31.9
2	21.64	37.09	15.45
3	22.57	38.76	16.19
Average:			21.18
<u>INTERMEDIATE</u>			
1	24.22	52.10	27.88
2	19.37	39.62	20.25
3	22.24	39.68	17.44
Average:			21.86
<u>HOT</u>			
1	--	--	--
2	22.83	25.14	3.03
3	19.75	32.46	12.71
Average:			7.87

^a These are the samples where lime was added in their respective clarification process.

Effect on Silicate Levels

Previous research (Muller, 1921) on hot liming systems conducted in the laboratory suggested that preheating of the juice before adding lime caused the precipitation of silicate. This would have an enormous impact in the factory as silicate contributes largely to the scaling in evaporators. Consequently, we decided to measure silicate levels in composite samples taken across the different clarification systems and results are listed in Table 2. Juice was preheated before liming only in the hot and intermediate clarification systems, however, silicate levels in the heated juice sometimes decreased as well as increased. Furthermore, there were no significant differences amongst silicate values in the final evaporator syrups for any of the three clarification systems. However, this may be because not all the mixed juice was preheated before intermediate and hot liming and measurements in three composite samples across the grinding season may not properly reflect real factory effects.

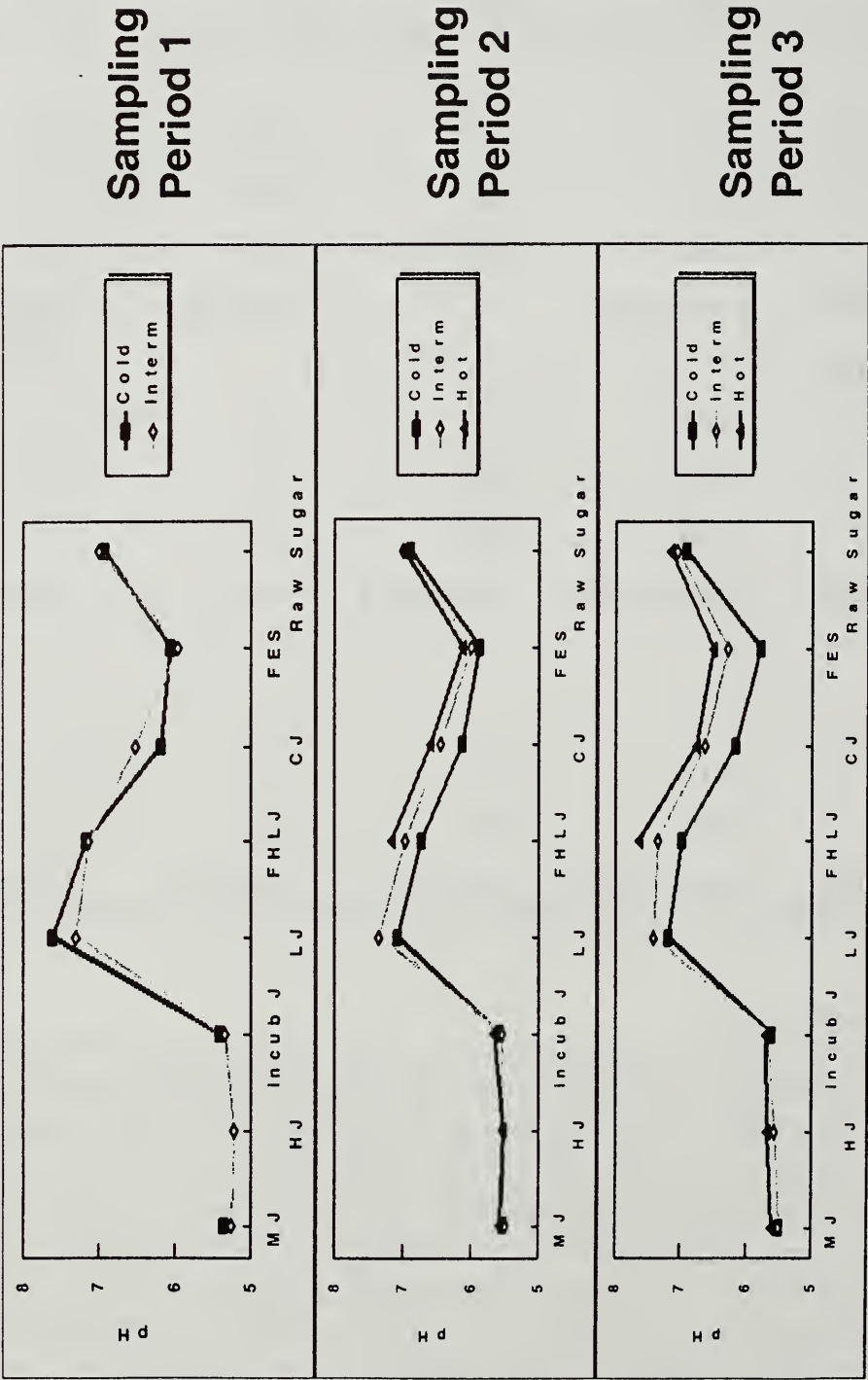
Table 2. Silicate concentrations in composite samples.

Clarification Process / Sampling Period	Si as Silicate (ppm/Brix basis)			
	MJ	HJ	CJ	FES
<u>COLD</u>				
1	51	n/a	6.7	9.6
2	91.4	n/a	10.2	11.0
3	138	n/a	9.9	9.5
Average ± Std. Dev	93.5±43.5		8.9±1.9	10.0±0.8
<u>INTERMEDIATE</u>				
1	48.3	59	8.7	10.0
2	310	247	7.5	13.4
3	109	127	10.4	12.1
Average ± Std. Dev	155.8±137.0	144.3±95.2	8.9±1.5	11.8±1.7
<u>HOT</u>				
1	--	--	--	--
2	115	107	13.5	10.3
3	96	104	14.8	10.3
Average ± Std. Dev	105.5±13.4	105.5±2.1	14.2±0.9	10.3±0.0

pH and Sucrose Loss Control

Changes in pH across each process and sampling period are illustrated in Figure 4. Patterns of changes in sample pH across each process were similar to those previously reported (Eggleson 2000a, Eggleson et al, 1991, 2002). In general, the pH of the CJs, FESs, and raw sugars were slightly higher in intermediate and hot liming than cold liming, which affected sucrose losses and profits (see Table 3). In the third sampling period at the end of the season, the effect of a higher target pH for the final evaporator syrup (FES) was studied in order to evaluate the impact on sucrose losses and economical profits. Across the 2000 grinding season the factory target pH of the FES was 6.0 but in this study it was increased to pH 6.3 in the final sampling period for both intermediate and hot liming (the effect was not studied on cold liming because of the clear disadvantages of operating this process). As can be seen in Figure 4, increasing the target FES pH caused pH increases in the FHLJ and subsequent samples; furthermore, sample pHs were slightly higher in hot liming than in intermediate liming even though less lime had to be added.

Fig. 4. Variations in pH amongst the three clarification systems^a



^a The target pH in sampling period 3 for intermediate and hot liming was increased from the usual value of pH 6.0 to 6.3

Table 3a. % Sucrose losses across the three clarification processes

Sampling Period	Clarification Process	% Average Sucrose losses ^a				
		Across the Incubator Tank	Across the Flash Heater	Across the Clarifier Tank	Across the Evaporator Station	Total
1	Cold	0.0	0.64	0.55	1.03	2.22
	Interm	0.0	0.33	0.19	0.90	1.41
	Hot	--	--	--	--	--
2	Cold	0.0	0.25	0.71	0.23	1.19
	Interm	0.0	0.0	0.88	0.32	1.2
	Hot	0.25	n/a	0.41	0.64	1.30
3	Cold	0.0	0.40	0.42	0.25	1.07
	Interm	0.0	0.40	0.46 ^b	0.09 ^{b,c}	0.55 ^b
	Hot	0.0	n/a	0.11 ^b	0.0 ^{b,c}	0.11 ^b

^a Sucrose losses calculated according to the formula in the Experimental section

^b The target pH of the final evaporator syrup was increased from 6.0 to pH 6.3

^c These figures do not take into account any glucose formed from inversion in pre- and intermediary evaporator bodies, which may have been further degraded or removed by precipitation causing reported sucrose losses across the evaporator station to be underestimates.

Table 3b. Season average U.S. dollar losses across the three clarification processes

	Clarification Process	Lost U.S. Dollar Profits ^c				
		Across the Incubator Tank	Across the Flash Heater	Across the Clarifier Tank	Across the Evaporator Station	Total
Season Average	Cold		\$176,920	\$222,178	\$209,834	\$608,932
	Interm		\$94,640	\$213,949	\$176,920	\$485,509
	Hot	\$18,515	n/a	\$181,034	\$126,724	\$326,273

^c U.S. dollar losses were calculated taking into account the lbs of raw sugar produced by the factory in 2000, the average cane sugar recovery rate, the average sucrose content of the raw sugar, and the current average price of raw sugar (19c per lb).

351 No sucrose losses were detected by the pre-heating of acidic MJ in intermediate and hot liming. Even though juice retention time was 8 min in the cold and intermediate incubation tank, no sucrose losses were detected (Table 3a), but there was a slight amount of sucrose lost in the hot lime incubation tanks where the retention time was 4 min longer at 12 min. This suggests that 12 min retention time may be too long and that 8 min should be adequate. A marked amount of sucrose (range 0.25-0.64%) was lost across the flash heater in cold liming (Table 3a) which caused a season average loss in revenue of US\$176,920 (Table 3b). In contrast, because of the design of the hot lime clarification process (see Figure 1a), losses across the flash heater were not applicable, and sucrose losses across the flash heater in intermediate liming were only detected in the first and final sampling periods (Table 3a). These differences may have been because of the slower settling in cold liming (see settling section), and the extra sucrose losses across the cold lime flash heater are an obvious disadvantage.

The effect of increasing the FES target pH from 6.0 to 6.3 for both intermediate and hot liming in sampling period 3, markedly decreased sucrose losses and increased economic profits, particularly across the evaporator station (Table 3). Furthermore, this positive effect was stronger for hot rather than intermediate liming. One of the reasons raw sugar factory staff are reluctant to increase either the target pH of the clarified juice or final evaporator syrup because they believe the extra lime required would increase evaporator scaling, particularly in the later evaporator bodies. However, the majority of unwanted scaling, especially in the U.S., is usually due to the precipitation of insoluble silicates, which are mostly associated with cane, soil, and trash entering the factory. Moreover, the markedly less lime required in hot liming would offset the additional lime required to increase the pH and improve sucrose inversion losses across the evaporators.

Overall across the season (Table 3b), the use of hot liming approximately saved the factory nearly half of the profits they would have lost if they operated cold liming. Total season losses in profits (Table 3b) for intermediate liming (US\$485,509), were better than in cold liming (US\$608,932) but worse than in hot liming (US\$326,273).

Turbidity Removal and Settling Performance

On season average, there was approximately 2.3% (significant at $P < .05$) more turbidity removal (MJ to CJ) in both hot and intermediate liming than in cold liming (see Table 4). This was slightly lower than the 4.6% difference observed by Eggleston et al (2002) between intermediate and cold liming, across the 1999 grinding season. The lower removal in this study may be because of different cane quality, as the factory processed considerably more green than burnt billeted cane than in the previous year, which increases the load of impurities. Turbidity values for clarified juices in both intermediate and hot liming were significantly ($P < .05$) lower than in cold liming (Table 4) and this was further reflected in the final evaporator syrup and raw sugar turbidity values (Table 4). Turbidity control was also markedly better in the hot liming FES and raw sugars.

Table 4. Season differences in turbidity values and removal^a

Sample	Turbidity at 420nm (ICU) ^b		
	Cold	Intermediate	Hot
MJ	57153 ± 10959a ^c	60283 ± 8014a	59437 ± 5504a
CJ	3165 ± 454a	1966 ± 354b	2100 ± 333b
FES	6079 ± 911a	5022 ± 762b	4868 ± 358b
Raw Sugar	755 ± 252	693 ± 311	445 ± 24
Av. % Turbidity Removal: MJ to CJ	94.5	96.7	96.5

^a N=18 except for hot liming where N=12^b Season average data presented with standard deviations^c Lower case letters represent statistical differences (P<.05) between the three clarification processes for season averages

The similarity in turbidity removal for hot and intermediate liming was expected because both processes had 50% of the MJ pre-heated before incubation. It is well known that the preheating of cane juice increases floc size through coagulation (see Eggleston et al, 2002), and larger flocs settle faster. However, differences were apparent in the settling performance of the flocculated limed juices from the three clarification processes (Table 5). Across the season, flocs were generally large in intermediate liming, compared to moderately large in hot liming and medium to small, fine flocs in cold liming (Table 5). The larger flocs in intermediate liming caused settling to be faster, as indicated by the higher initial settling rates and lower break point times (Table 5). Reasons for the larger flocs in intermediate compared to hot liming are not clear; the only differences between the two processes were the higher temperature and lower retention time of lime addition in the hot liming. The addition of lime at higher temperatures and for a shorter time in hot liming may have ruptured some of the flocs formed previously on pre-heating the MJ. Another explanation could be that the formation of calcium phosphate precipitate was lower in hot liming because of the shorter liming time. Overall, results strongly suggest that the pre-heating of juice is a large contributor to settling performance and probably contributes more to what was previously considered (Simpson, 1996).

Effect on Polysaccharide Concentrations

The two major polysaccharides which can profoundly impact cane processing are dextran and starch. Dextran is formed from cane contamination with *Leuconostoc* bacteria. Starch is present in the cane as a storage source and is less abundant in mature than immature cane.

Dextran removal and formation (Table 6), in general, followed previous observations by Eggleston et al (2002). One of the advantages of preheating MJ before liming, as in intermediate and hot lime processes, is the removal of polysaccharides including dextran, which also cause the levels to be lower in the incubated juice. However, a marked drop of dextran was also noted in the “cold” lime incubation tank, which suggests that some precipitation occurred with color removal. As expected, dextran was markedly formed in the cold lime tanks. Dextran concentrations across the intermediate lime process were not as good as in the previous season, but hot liming levels were much better

although it must be pointed out that the incoming MJ levels were better too.

As expected, starch decreased across the season for all samples and clarification systems, because of the increased maturity of cane being processed. MJ starch varied little for the three clarification processes (Figure 5). In both intermediate and hot liming, starch was removed in the HJ most likely because of precipitation. Starch was also degraded in the incubator tank for both intermediate and hot liming (Figure 5) which is most likely because the addition of recycled filtrate from the clarifier reduced the MJ acidity, enabling the natural juice diastase to degrade starch (see Eggleston et al, 2002). Starch increased for all three processes in the clarified juice because of solubilization and gelatinization and decreased in the FES because of the factory application of commercial α -amylase.

Table 5. Settling characteristics of the three clarification processes^a

Clarification Method	Sampling Period	ISR (ml/min)	Break Point ^b (secs)	MV _{inf} (ml)	Floc characteristics
Cold					
	1	94.3	41.3	13.9	Medium to large flocs
	2	108.3	41.2	12.1	Small, fine flocs
	3	95.7	43.5	14.5	Small, fine flocs
Intermediate					
	1	115	39	10.1	Mostly large flocs
	2	140	33.3	9.6	Large flocs
	3	180 ^c	30.2	10.7	Large to v. large flocs
Hot					
	1	--	--	--	--
	2	103.8	43.3	12.2	Moderately large flocs
	3	91.4 ^d	53.5	12.0	Medium to large flocs

^a Averages \pm standard errors; $N \leq 6$

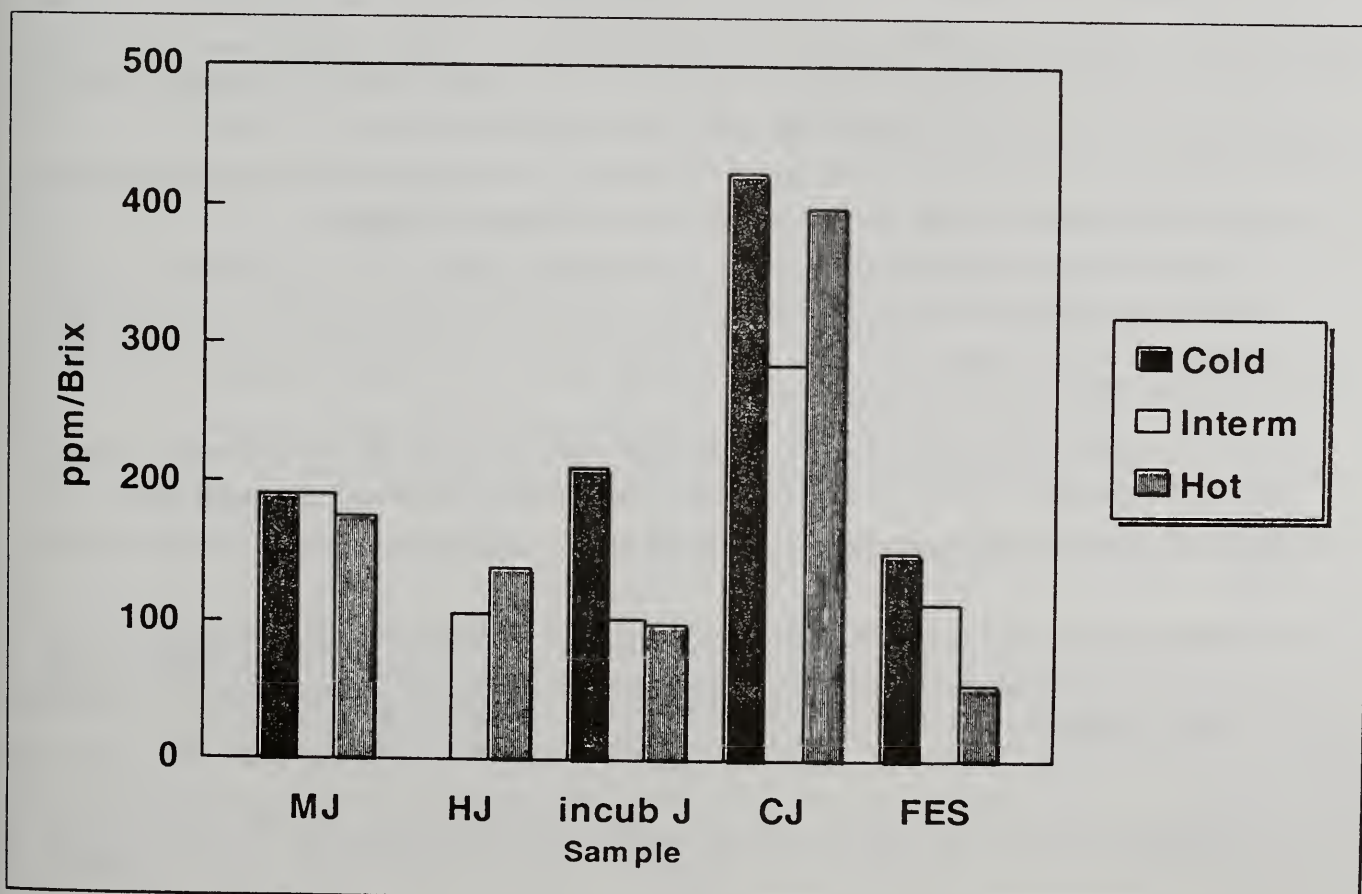
^b Break Point was the time in seconds it took for the flocculated, flash heated limed juice to settle to half its original volume

^c $N \leq 4$

^d $N \leq 2$ due to difficulty of measuring settling in some samples

Table 6. Season average dextran (ASI-II method) data^a

Sample	Dextran (ppm/Brix) ^b		
	Cold	Intermediate	Hot
MJ	790 ± 480a ^c	804 ± 513a	551 ± 136a
HJ	N/A	459 ± 277a	400 ± 185a
Incub J	529 ± 317	717 ± 539	582 ± 187
LJ/HLJ	1001 ± 550a	680 ± 451a	N/A
FHLJ	362 ± 112a	697 ± 716a	361 ± 118a
CJ	357 ± 183a	492 ± 483a	295 ± 140a
FES	467 ± 172a	582 ± 428a	274 ± 65a
Raw Sugar	432 ± 315	356 ± 88	158 ± 74

^a N=18 except for hot liming samples where N=12^b Season average data presented with standard deviations^c Lower case letters represent statistical differences (P<.05) between the three clarification processes for season averages**Figure 5. Variations in starch concentrations**

CONCLUSIONS

For most clarification parameters investigated, both hot and intermediate liming performed much better than cold liming, and hot liming generally offered some extra advantages over intermediate liming. Most of the advantages offered by hot and intermediate liming were because of 1) pre-heating the juice before incubation and liming which markedly improves impurity removal, and 2) in the case of hot liming, the much lower consumption of lime. It is not, therefore, necessary to gain these advantages by having an incubator tank. However, an incubation tank does offer some benefits such as increased starch removal and stabilization of factory juice flow throughput. It is also important to point out that, in hot liming, the lime does not have to be added after the flash heater, but can be added just before the heater and this could increase mixing. In South Africa, many factories add lime upstream of the flash tank in an in-line static mixer (Meadows, 1996).

- ▶ Less sucrose was lost to inversion reactions across both hot (season av. 0.79%) and intermediate (0.97%) lime processes than across cold liming (1.48%). By operating hot liming, the reduction in sucrose losses alone saved the factory approximately US\$283,000 over cold liming.
- ▶ Increasing the factory target pH of the final evaporator syrup (FES) from ~pH 6.0 to 6.3, in sampling period 3 in both hot and intermediate liming, caused a very marked reduction in sucrose inversion losses, particularly across the clarifiers and evaporators.
- ▶ Dramatically less lime had to be added in hot liming compared to either cold or intermediate liming, with the factory consuming across the 2000 grinding season, on average, only 1.01 lbs lime/ton cane compared to 1.28 for the 1999 grinding season when intermediate rather than hot liming was operated. Furthermore, even across the next grinding season in 2001, when the factory operated hot liming and increased the target FES pH to 6.3 from 6.0 in the previous season, 1.05 lbs lime/ton cane were still only consumed.
- ▶ Pre-heating 50% of the MJ in both intermediate and hot liming consistently removed color, dextran, and starch, but silicate levels were not significantly changed.
- ▶ Although, the fastest settling occurred in intermediate liming, ~ 2.1% (season av.) more turbidity removal (MJ to clarified juice CJ) occurred in both hot and intermediate liming compared to cold liming, with better CJ turbidity control. Subsequent FES and raw sugar turbidity values were better in hot liming.
- ▶ Markedly less color (~2.5%) formed on hot liming because of reduced retention time of liming, compared to ~17% color formation in cold and intermediate lime clarification.
- ▶ Dextran removal was best across hot liming and, as expected, dextran formed in the cold lime tanks.
- ▶ Using hot liming across the season, the factory observed 12-15% more heating capacity in the limed juice heat exchangers and a 90% reduction in the quantity of chemicals needed to clean the heat exchangers.

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POTENTIAL NEW PRODUCT DEVELOPMENT USING MEMBRANES

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ABSTRACT

Membrane treatment of sugar processing streams holds great promise in reducing energy usage, reduction or elimination of chemical clarification and improved final product quality. Another promising area of application is in production of new products, such as organic sugars, or products from unusual sources in the process, such as the recovery house. The recovery house is an important area of the refinery, but one in which low purity samples are recycled back into the process to recover sugar, with the added expense of increased clarification demands and the introduction of additional impurities. A series of tests were undertaken using a Koch spiral membrane system to treat the various streams of the recovery house of a local refinery. Removal of total polysaccharides, starch, dextran, turbidity and color were noted, averaging 4 to 38% color removal, 93% turbidity removal, 73% starch removal, 63% dextran removal, and 73% total polysaccharide removal. The permeates from soft syrup (color >12,000 ICU) and affination syrup (color >20,000) were further processed into crystalline products. These "soft light" sugars, color < 1000 had very pleasant aromas and appearance. The sensory characteristics of these new products are described.

INTRODUCTION

The sugar refining process tries to recover all usable sugar with the least possible amount of loss to molasses. The final processes for recovering sugar occur in the Recovery House, an often little understood and sometimes neglected part of the refinery. The streams in the Recovery House are low purity and high in color and macromolecules, as well as turbidity. About 10% of the solids in raw sugar in the refinery goes to affination syrup. The quality of the affination syrup is largely a function of the quality of the raw sugar. In some refineries, part of the affination syrup is processed into soft sugar (brown sugar) after dilution and clean-up. Excess affination syrup goes to the Recovery House. In refineries without soft sugar production, all of the affination syrup goes to boil remelts. A high remelt is boiled first, with the syrup from this strike boiled back in one or two stages into low remelts. The runoff from the last boiling is the final molasses. The remelt sugars are sent to the clarifiers.

Little has been written about the recovery house. The Cane Sugar Handbook devotes only a short section to it (1). In 1987 and again in 2000, SIT had symposia on affination and recovery operations (2, 3).

Remelt sugars are considered non-food grade due to their low purity, high color and ash, and generally harsh flavor. Affination syrup can have a high microbiological load, since it has not been subjected to the elevated heat of other refinery processes. For this reason, remelts and low purity streams undergo rather extensive extra processing before being recycled back into the main stream. Remelt sugars are the source of recycled impurities, an undesirable state of affairs in the refinery. It was, therefore, of interest to examine membrane processes in the recovery house, as a sort of one-step clean-up process, which could be applied to produce higher quality, food-grade products, possibly conferring an economic advantage to the refinery.

Membrane processes have a long history of study and tremendous potential in the cane and beet sugar industry (4-11, as examples). Streams studied for membrane application have included clarified cane juice, molasses, beet syrup, raw cane sugar and used brine from decolorizing resins.

MATERIALS AND METHODS

Experimental Protocol

Experiments were carried out at a local refinery. Recovery house streams were treated with a Koch spiral polymeric membrane (HFM-116) having a nominal molecular weight cut-off of 50,000 Daltons. The streams available for membrane processing were affination syrup, soft syrup, high remelt runoff, high remelt wash, remelt runoff and remelt wash. In this refinery, remelt wash and remelt run-off are combined to form a stream called repurge, due to the small volumes of these streams.

The feed material was diluted to 25-27 Brix and heated to 80°C in tanks. Material was fed into the membranes with treatment continuing for 3-4 hours. Samples taken included the undiluted material, the diluted feed material, the final permeate and the retentate. Parameters for the Recovery House samples were chosen based on previous experience with clarified cane juice and B-molasses. B-molasses has a composition similar to that of Recovery House streams. Because of equipment limitations, it sometimes proved difficult to maintain the target sample temperature of 80°C, with it falling to as low as 70°C during some of the experiments.

Analyses of samples included characterization of the undiluted recovery house stream material, the diluted feed, the permeate and the retentate. Samples were analyzed for color, turbidity, ash, total polysaccharides, dextran, pH, Brix, purity, and invert.

Because these streams were being considered as a new source for potential food products, particularly as flavorful syrups or sugars, it was also of interest to test the volatiles in them to determine if there are desirable flavor and odor compounds in these samples. Analysis of the volatiles in the recovery house streams was carried out by SPME/GC/MS (solid phase microextraction - gas chromatography - mass spectrometry).

Sugar was crystallized from one soft syrup permeate and four affination syrup permeates. The permeates were evaporated to syrup and crystallized at the pilot facilities of the Audubon Sugar Institute.

RESULTS

Table 1 presents the membrane performance parameters with water, compared to the low purity samples. The difference in performance is, of course, due to the impure nature of the materials, and the added viscosity caused by sucrose, polysaccharides and turbidity.

Table 1. Membrane performance parameters, water and test samples

Feed	P _{in} , psi	P _{out} , psi	TMP, psi	Flux LMH	Feed Rate, lpm	Recirc. Rate, lpm	Tank Temp °C	Recirc Temp °C	CF
Water	19	2.8	10.8	163	10	260	80	90	200
Sample	50+	47+	50-55	40-50	4	230	80	90	5

Table explanation:

TMP = transmembrane pressure

LMH = L/m²/hr

lpm = liters per minute

CF = concentration factor

Composition of the Feed Material

Table 2 gives a “snapshot” of the composition of each the recovery house streams. The feed material can be expected to change from day-to-day, depending on the raw sugar being processed, but the data in Table 2 are expected to be typical. The recovery house samples range from 70-78 brix; with a pH in the 6.5 to 6.65 range; they are fairly low in purity; moderately high in invert and ash; and have uniformly high color, turbidity and total polysaccharide levels. Starch and dextran are two known components of the total polysaccharide composition, but they account for less than 20% of the total polysaccharides in the samples. The remaining polysaccharides are composed of various polysaccharides from the sugarcane plant, the most important being the soluble cell wall indigenous sugarcane polysaccharide (ISP). An average starch level of around 500 ppm and dextran level of 1000-1400 is expected. The very high dextran level of the soft syrup sample is noted; this may represent the development of a dextran problem in this stream or a very high dextran input from the raw sugar. Dextran levels this high are unusual.

Volatiles in remelt samples. SPME/GC/MS showed the presence of numerous agreeable flavor compounds, including those responsible for nutty, baked/browned aromas (pyrazine derivatives), and caramel aromas (furanone derivatives). Also present were vanillin (vanilla) and benzaldehyde (almond flavor). This would indicate that pleasant aromas would be present in the final product, as membrane treatment does not remove small, volatile molecules.

Table 2. Composition of Recovery House Streams -- "A snapshot"

Parameter	Affination Syrup	Soft Syrup	High Remelt Runoff	High Remelt Wash	Remelt Runoff	Remelt Wash
Brix	73.5	70.2	74.8	77.1	78.5	75.4
pH	6.39	6.49	6.65	6.63	6.58	6.54
Purity	83.4	78.6	57.3	63.9	68.4	77.4
Invert	3.00	6.71	8.23	5.56	5.79	4.50
Color	61,667	31,000	141,750	136,250	129,250	91,000
Turbidity	10,666	4,667	28,250	26,250	24,500	15,750
Total Poly	7,610	19,245	22,543	17,609	16,769	12,306
Dextran	663	7589	841	1155	1467	1415
Starch	259	438	618	600	559	485
Ash	2.89	5.97	9.03	7.38	7.11	5.29

Table explanation:

Purity, Invert and Ash are reported as % dry solids

Color and Turbidity are reported as ICUMSA Color Units (IU)

Dextran, Starch and Total Polysaccharides are reported as ppm on solids

Microbiological testing. Microbiological testing was carried out on the soft syrup, with promising results. The soft syrup feed had 130 cfu (colony forming units)/ml and 210 cfu/ml respectively in feed 1 and feed 2, while the permeate was negative for mesophiles and coliforms. This indicates that the high temperature of processing, along with the membrane treatment was able to significantly reduce the microbiological load. Microbiological testing of the affination syrups boiled into sugar is shown elsewhere in this report.

Affination syrup. Analyses of the products are shown in Table 3. The starting brix was 75, with dilution to an operating brix of 27. The experiment lasted about 3 hours. Inlet pressure began at 61 psi, rising to 70 psi at the end of the run. Transmembrane pressure also increased, from 52 psi to 62 psi. Concentration factor and feed rate remained close to the set-point throughout, 5.0 and 3.5 lpm, respectively. Flux rate declined steadily from 48 to 36.

There was significant removal of color (41.7%), turbidity (90.6%), starch (72.2%), dextran (32.3%) and total polysaccharides (66.6%). There was little change in pH, ash and invert.

Soft syrup. Analyses of the products are shown in Table 4. The starting brix was 71, with dilution to an operating brix of 24. The experiment lasted about 4 hours. Inlet pressure remained steady at 23-24 psi, as did transmembrane pressure, at 13.4-13.8. However, flux rate declined steadily from 61.2 to 49.4. A feed rate of 5 lpm was originally set, but this could not be achieved so it was dropped to 4 lpm. Concentration factor, set at 5.0, also fluctuated somewhat

There was significant removal of turbidity (86.5%), starch (61.3%), dextran (88.7%) and total polysaccharides (79.5%), but only a small amount of color was removed, 3.4%.

High remelt wash. Analyses of the products are shown in Table 5. The starting brix was 76.7, with dilution to an operating brix of 25. The experiment lasted about 3 hours. Inlet pressure began at 57 psi, rising to a high of 66 psi, then dropping to 59 as the feed rate fell. Transmembrane pressure followed a similar pattern as well as flux rate. There was difficulty with reaching operating temperature in the tank, and it is felt that, along with the highly impure nature of the feed material, the colder temperature contributed to rapid fouling of the membrane.

There was significant removal of color (34.0%), turbidity (98.9%), starch (75.4%), dextran (48.1%) and total polysaccharides (71.9%).

High remelt runoff. Analyses of the products are shown in Table 6. The starting brix was 76, with dilution to an operating brix of 25. The experiment lasted about 4 hours. There was also difficulty in this experiment to attain and maintain the desired temperature of the feed, which, along with the highly impure nature of the material, contributed to poor performance, and the inability to maintain a good feed rate. Transmembrane pressure increased from 51 to 58 psi. Flux decreased from 53 to 40 lmh. Inlet pressure began at 63.7 and rose to 66.6 at the end. There was a system shut-off 2 hours and 40 minutes into the run due to high recirculation temperature.

Color (37.7%), turbidity (92.2%), starch (77.5%), dextran (57.8%) and total polysaccharides (69.0%) were significantly removed.

Repurge (remelt runoff + remelt wash). The data are not shown for repurge. The starting brix was 71, with dilution to an operating brix of 27. The experiment lasted about 3 hours and 20 minutes. Inlet pressure increased from 20 psi to 48 psi; TMP rose from 11 to 39 psi; but flux rate was more-or-less steady, fluctuating between 49-46 lmh. Feed rate of 4 lpm could not be obtained, rather it hovered around 3.7 lpm. Concentration factor remained within the range of the set point of 5.0. Part of the difficulties experienced with this run was the low steam supply, which meant lowering the recirculation flow rate from 250/260 to 230 lpm to keep the system working.

Turbidity (95.0%), starch (79.9%), dextran (90.5%) and total polysaccharides (77.1%) were significantly removed. Only 3.5% of color was removed.

Table 3. Membrane treatment of affination syrup

Sample	Time, hr.	Flux	Brix	pH	Purity	Color	Turbidity	Ash	Invert	Polys	Dextran	Starch
Feed	1:15	39.0	27.9	7.18	88.60	43,224	6593	3.55	2.72	5,897	340	730
Perm-1	1:15	---	26.8	7.11	89.21	24,810	535	3.56	2.83	2,087	265	176
Ret-1	1:15	---	29.2	7.20	---	82,587	31,400	3.49	2.62	---	---	---
Feed	2:15	36.8	26.5	7.13	88.94	41,385	7874	3.62	2.72	5,976	426	510
Perm-2	2:15	---	26.2	7.05	87.33	24,504	840	3.62	2.86	1,884	245	160
Ret-2	2:15	---	28.3	7.20	---	85,075	37,460	3.51	2.56	---	---	---

Table 4. Membrane treatment of soft syrup

Sample	Time, hr.	Flux	Brix	pH	Purity	Color	Turbidity	Ash	Invert	Polys	Dextran	Starch
Feed-1	1:00	51.3	24.3	6.65	86.58	16,459	2,410	4.32	4.63	12,643	4,760	494
Perm-1	1:00	---	25.1	6.61	84.82	15,877	332	4.35	4.62	2,868	580	227
Ret-1	1:00	---	26.4	6.84	---	18,712	17,944	3.97	4.29	---	---	---
Feed-2	2:45	47.3	26.4	6.68	87.12	14,264	2,300	4.02	4.24	11,635	4,156	532
Perm-2	2:45	---	26.2	6.65	85.59	13,804	306	4.07	4.37	2,126	435	167
Ret-2	2:45	---	27.3	6.75	---	16,948	10,736	3.87	4.10	---	---	---

Table 5. Membrane treatment of high remelt wash

Sample	Time, hr.	Flux	Brix	pH	Purity	Color	Turbidity	Ash	Invert	Polys	Dextran	Starch
Feed-1	0:40	40.4	24.3	6.16	62.50	131,000	19,807	8.77	8.28	17,780	593	1535
Perm-1	0:40	---	23.4	6.08	66.06	100,714	237	7.75	8.56	5593	338	431
Ret-1	0:40	---	27.6	6.28	54.99	290,666	191,942	8.97	7.08	---	---	---
Feed-2	2:40	32.2	24.6	6.20	62.75	150,385	20,961	8.84	8.22	19,311	745	1625
Perm-2	2:40	---	23.4	6.10	65.15	83,043	217	8.60	8.71	4769	349	355
Ret-2	2:40	---	28.0	6.26	53.61	328,571	157,143	7.69	6.98	---	---	--

Table 6. Membrane treatment of high remelt run-off

Sample	Time, hr.	Flux	Brix	pH	Purity	Color	Turbidity	Ash	Invert	Polys	Dextran	Starch
Feed-1	1:00	---	25.0	6.35	53.12	116,500	20,000	11.27	7.72	19,799	1,074	2,311
Perm-1	1:00	48.6	24.2	6.43	54.78	74,000	2,000	11.70	8.03	6,150	516	455
Ret-1	1:00	---	29.0	6.77	46.68	199,000	79,500	10.03	6.64	56,023	---	6,077
Feed-2	2:00	---	24.1	6.47	53.22	110,500	19,500	11.55	7.73	20,616	2,495	1,933
Perm-2	2:00	45.4	23.3	6.37	55.02	73,000	1,000	11.87	8.16	6,281	375	438
Ret-2	2:00	---	28.5	6.79	50.13	259,500	107,500	9.98	6.31	65,798	---	6,369
Feed-3	4:00	---	24.3	6.59	51.29	122,000	24,500	11.46	7.81	21,942	1,491	1,982
Perm-3	4:00	40.2	23.2	6.36	54.27	70,000	2,000	11.85	8.18	6,875	948	500
Ret-3	4:00	---	28.5	6.75	48.63	226,500	98,500	9.70	6.37	90,947	---	8,229

DISCUSSION

The materials in the Recovery House are varied in composition and will change from day-to-day, but all are characterized by low purity and high color, turbidity, polysaccharides, ash and invert. Of the streams studied, the most promising for new food product development are affination syrup and soft syrup. Soft syrup is particularly easy to process by membranes because it has already been filtered through char, thus much of the insoluble material (turbidity) has already been removed. Table 7 summarizes the removal of macromolecules from the various streams in the Recovery House.

Table 7. Summary of macromolecule removal by Koch spiral polymeric membrane HFM-16, 50 kD. Figure shown represent % removed from feed material, based on permeate analysis.

Sample	Color	Turbidity	Starch	Dextran	Total Polys
1. Affination	41.7	90.6	72.2	32.3	66.6
2. Soft syrup	3.4	86.5	61.3	88.7	79.5
3. Hi remelt wash	34.0	98.9	75.1	48.1	71.9
4. Hi remelt runoff	37.7	92.2	77.5	57.8	69.0
5. Repurge	3.5	95.0	79.9	90.5	77.1
Mean removal	3.5/37.8	92.6	73.2	63.5	72.8

Not unexpectedly, the best performance of the membrane is in the area of turbidity removal, averaging 92.6% removal of turbidity. The removal of starch, dextran and total polysaccharides is also high -- averaging 73.2%, 63.5% and 72.8% respectively. The removal of dextran was not as consistent as it was for starch and total polysaccharide, reflecting differences in the molecular weight range of the dextran in the various streams. The lesser removal of dextran in affination syrup and high remelt runoff could reflect input raw sugars with a lot of low molecular weight dextran, possibly resulting from the use of dextranase in the mill. Since samples were processed one week at a time, each stream could easily represent different input raw sugars.

The removal of color was bimodal, an unexpected result. Soft syrup and repurge had less than 5% removal. (In an earlier study on B molasses in a cane mill, we had obtained only 4% color removal in two experiments, unpublished results.) The other streams averaged about 38% color removal. In earlier studies at SPRI, in gel permeation studies of the high molecular weight colorant in raw sugar (>12,000 Daltons) it was noted that the highest molecular weight colorants had less color than the intermediate and lower molecular weight colorants. The results with this work show that there are different molecular weight distributions in the various recovery house streams, reflecting the different treatments they have undergone. Purity, invert and ash were not significantly changed by membrane processing, which is to be expected.

A soft light sugar from soft syrup. The membrane treated soft sugar was evaporated to a syrup and crystallized, producing a pleasant, pale "soft" sugar, with a color of 800 ICU, 0.137% moisture and 0.15% ash. This sugar is shown in Figure 1. The taste of the product was clean and sweet with mild caramel tones. There were no harsh flavor notes. The harsh flavor of the recovery house samples is felt to be due to the highly polymerized large colorant molecules and high ash content. This study has shown that membrane treatment at a 50,000 Dalton cut-off is able to remove all traces of the harsh flavor. A micrograph of the crystals is shown in Figure 2. There were some elongated crystals and some agglomeration.



Figure 2. Soft light sugar boiled from membrane treated soft syrup.

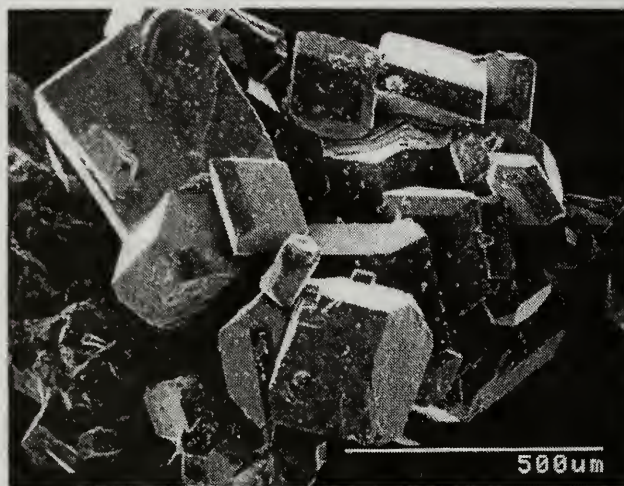


Figure 3. Micrograph of crystals boiled from membrane-treated soft syrup. (Magnification = 100X.)

Sugars boiled from affination syrup. In another set of experiments, four batches of affination syrups were membrane treated, concentrated to syrup and boiled. Two batches of affination syrup were treated with 500 ppm hydrogen peroxide prior to membrane treatment to see if further color improvement would be obtained. However, since the pH was not adjusted upward, the hydrogen peroxide had no effect. The ranges of color, polysaccharide, turbidity and ash in the affination syrups prior to treatment are shown in Table 8a. In Table 8b is shown the composition of the corresponding permeates.

Table 8a. Composition of affination syrups prior to membrane treatment.

Sample	Purity	Color	Turbidity	Ash, %	Polys, ppm
1	85.80	61,860	7075	3.17	8549
2	85.95	25,760	7490	4.06	6499
3	82.88	22,500	8200	4.27	6348
4	84.68	26,022	8714	3.20	4241

Table 8b. Composition of affination syrups permeates after membrane treatment.

Sample	Purity	Color	Turbidity	Ash, %	Polys, ppm
1	89.30	27,655	580	3.51	2005
2	88.55	21,548	444	4.52	3278
3	82.84	17,845	558	4.76	2548
4	83.92	16,716	480	3.58	2150

Microbiological testing of affination products. Microbiological testing for total aerobic plate counts (APC), coliforms, yeast and blue mold were performed on three of the affination treatment sets. The results are shown in Table 9. While the affination syrups had a fairly high microbiological load, the heating of the feed caused a significant decrease, and the permeates were clean.

Table 9. Microbiological testing of affination products before and after membrane treatment

Sample	APC/g			Coliforms/g			Yeast/g			Blue Mold/g		
	1	2	3	1	2	3	1	2	3	1	2	3
Water	0	0	*	0	0	*	0	0	*	0	0	0
Aff'n.	550	1300	700	20	660	80	640	>1000	100	3	0	30
Feed	300	410	150	20	0	0	0	0	180	0	3	46
Perm.	0	3	10	0	0	0	0	0	0	0	1	0

* No sample provided for testing

The resulting sugars were different in aroma from the sugar produced with soft syrup, having a more distinctly raw sugar or "fresh cane" odor, whereas the sugar produced from membrane-treated soft syrup was more browned/brown-sugar like, reflecting its passage through the various refinery processes with further browning reactions taking place.

Table 10 shows the analysis of the four sugars boiled from affination syrup. Table 11 compares the permeate to the sugars produced. There was an average 96.5% color removal, 85% polysaccharide removal and 94.4% ash decrease.

Table 10. Composition of sugars boiled from membrane-treated affination syrup.

Sugar	pH	Color, IU	Turb, IU	Polys, ppm	Cond. Ash, %	Moisture, %	Purity	Invert %
1	6.31	610	106	20	0.03	0.401	99.52	0.94
2	6.83	765	148	226	0.11	0.144	97.12	0.16
3	6.84	974	228	158	0.21	0.438	99.04	0.42
4	6.68	458	59	89	0.06	0.896	95.13	0.33

Table 11. Comparison of affination permeate composition and sugar boiled therefrom.

Sugar	Color			Polysaccharide			Ash		
	Perm	Sugar	% change	Perm	Sugar	% change	Perm	Sugar	% change
1	27,655	610	97.8	2005	196	90.2	3.51	0.038	98.9
2	21,548	765	96.4	3278	765	76.7	4.42	0.687	84.8
3	17,845	974	94.5	2548	468	81.6	4.76	0.206	95.5
4	16,716	458	97.3	2150	182	91.5	3.58	0.062	98.3

The resulting sugars are a pale gold to beige in color with small crystals, a “soft” texture and mild aroma and flavor. This is a distinctly different product from the commercial brown/soft sugars available on the market in the United States and Canada, as seen by a comparison to the data in Table 12, which gives the analysis of some commercial brown sugars. The sugars from membrane treatment are lower in color, turbidity, ash, polysaccharides and invert, with a higher pH and purity. The flavor of the membrane sugars is also milder.

Table 12. Composition of some commercial brown sugars.

Sugar	pH	Color, ICU	Turb, ICU	Polys, ppm	Cond. Ash, %	Moisture, %	Purity	Invert %
Domino Brownulated	6.93	5733	3998	1625	1.39	1.17	92.13	0.60
Domino light brown	5.33	3841	382	1012	1.26	1.26	94.36	0.99
Domino dark brown	4.80	7789	10,172	1335	1.53	1.53	94.96	1.28

Edible Syrups from Membrane Processing.

Crystalline sugar need not be the only product from membrane treatment of recovery house streams. Edible syrup or concentrated permeate is another potential new food product, which could be used in various food processing applications, where high color, salt and flavor are required, such as in certain types of bakery, in sauces, condiments, and marinades. There would be a considerable energy savings in not taking the product all the way to the crystalline stage. Because these products are sometimes very high in ash, giving too salty a flavor for using alone as, for example a syrup for pancakes, they could be blended with white corn syrup or used as an ingredient in a barbecue sauce or a marinade.

Retentates. The retentates represent a challenge in membrane processing, and the goal is to have as little as possible remaining in the process by using maximum concentration. In this study, retentate represented 20% of the feed. The retentates were difficult to analyze. The analyses for purity and total polysaccharide, in particular, encountered a lot of interference, and for this reason were not done in most of the samples. The data in Tables 3-6 show that the retentates are highly concentrated in color, polysaccharide and turbidity. There appeared to be a slight decrease in ash and invert, compared to the feed, and a more significant decrease in purity, indicated by the available data. Since the same proportion of sucrose, invert and ash are expected in the retentate as the feed and permeate at the cut-off used, the decreases represent, not so much decreases in these constituents, but rather increases in the other impurities. Methods to recover sucrose from retentate by methods such as coagulation and precipitation of color and polysaccharide would be possible but expensive, and the best strategy would be to create as little retentate as possible.

CONCLUSION

This work has shown that the low purity streams from the recovery house can be successfully treated with membranes to remove turbidity, harsh flavor and macromolecules, leading to potential new products from this part of the refinery. The following membrane applications are potentially promising for recovery house materials.

(1) Treatment of affination syrup or soft syrup to produce a liquor from which can be boiled additional edible sugar or syrup. This would eliminate much of the remelt sugar output and would add to the economic feasibility of the process. It is also possible to envision combining the affination and soft remelt streams for the same purpose, therefore producing a larger quantity of additional high value sugar.

(2) Treatment of various low-purity run-off and wash streams to create syrups with potential application in food processing, especially for specialty breads and savory foods. Mixing the treated streams with other types of sugar and syrup could produce tailor-made materials for various food applications. Additionally, the highly colored, cleaned up syrups may have potential for food color or caramel. The fact that no chemical processing was undertaken to produce the color is an advantage, in that the food can have a "clean label."

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COMPOSITION OF EVAPORATOR SCALE IN LOUISIANA MILLS

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ABSTRACT

Samples of evaporator scale were obtained from cane mills in Louisiana, as well as scale scraped from the top, middle and bottom of a set of tubes, one from each effect, from one mill. EPA Method 3050B (Acid Digestion of Sediment, Sludges and Soils) was adapted for digesting the samples. Digested samples were analyzed by inductively coupled plasma (ICP) for calcium, magnesium, sulfur, phosphorus, copper, nickel, aluminum and iron. Silica was semi-quantitatively determined gravimetrically by weighing the residue remaining after digestion. There was good agreement between commercial testing using atomic absorption spectroscopy (AAS) and ICP results. The main components in all the Louisiana cane growing areas were calcium and silica. The proportion of scale components from different growing areas is presented.

INTRODUCTION

The problem of evaporator scale formation is severe in Louisiana, as it is in most cane processing areas of the world, requiring frequent shut-down of the factory for clean-out. Anti-scalants seem to help to a limited extent, and practices such as maintaining a regular juice level and keeping pH as high as possible, can help lengthen the time between boil-out. Only scale inhibitors based on acrylic acid (CFR 21:173.b(b)(1)) or maleic acid (CFR 21:173.45(b)) meet FDA approval. The type of scale will determine the cleaning regime -- acid for carbonate and phosphates scale, and alkali for silicate and sulfate scale, or combinations of acid and alkali. Researchers in South Africa and Australia have done a great deal of work to elucidate the nature of scale.¹⁻⁴

MATERIALS AND METHODS

Samples. In addition to the samples from the set of tubes described above, samples of scale were collected from mills throughout the Louisiana cane growing areas.

Digestion of Samples. The samples, about 0.5 g, were digested according to EPA Method 3050B (Acid Digestion of Sediment, Sludges and Soils), using nitric acid and hydrogen peroxide.

Inductively Coupled Plasma. Elements in the digested samples were determined by Inductively Coupled Plasma (ICP) using a Leeman Labs Profile Dual View (Leeman Labs, Hudson, NH, 03051). All elements were analyzed in axial mode. Wavelengths used to analyze samples were: Ni - 231.604; Cu - 324.75; Mg - 279.553; Ca - 317.933; P - 213.668; S - 180.669; Fe - 238.204; Al - 309.271.

Silica. Silica was determined semi-quantitatively by weighing the dried residue remaining after digestion.

RESULTS AND DISCUSSION

A mild digestion with trifluoroacetic acid (TCA) of several thoroughly washed samples showed the presence of citric acid (1.1-2.6%) and aconitic acid (0.5%) along with traces of glucose and fructose, findings which indicated that there was also a significant organic component to the scale, possibly acting as a matrix for deposition of the inorganic material.

Commercial analysis of one of the samples of evaporator scale is shown in Table 1. The results for calcium, sulfur, phosphorus and silica obtained by nitric acid/hydrogen peroxide digestion, followed by ICP analysis, are shown for comparison purposes. There is good agreement in the results. The loss on ignition is mostly indicative of organic material, and at 34.23% is substantial. Evaporator scale may consists of a matrix of organic material composed of organic acids, sugars, amino acids and polysaccharides, which may give the scale bulk and cohesiveness.

Table 1. Comparison of ICP results with commercial analysis of scale sample

<u>Analyte</u>	<u>Result (% of scale)</u>	<u>ICP results</u>
Silicate	10.62	10.75
Calcium	19.27	23.3
Sulfur	<0.05	0.67
Phosphorus	1.89	2.00
Carbonate	1.35	not done
Loss on ignition	34.23	not done

Louisiana has three distinct cane growing areas, described as the Bayou Lafourche area, the Bayou Teche area and the Mississippi River area. The appearance of the scale samples from the mills differed greatly, ranging from massive and crumbly, to rock-like, and in color from off-white to grey to dark brown. The appearance could not be correlated with a particular growing area.

The scale from the four effects and the pre-evaporator from one mill also showed different textures and colors from one effect to the other, as described in Table 2. As shown in the table, the abundance of the scraped material was variable, with the largest amount being scraped from the fourth effect and the next largest amount scraped from the pre-evaporator. Because of the scraping action, copper was also removed into the samples, so there is a correction made for the presence of copper in the reported calculations.

Table 2. Description of scale obtained from evaporator tubes from a Louisiana factory in the Bayou Teche area.

Sample	Description	Wt, g	Appearance
PT	Pre-evaporator, top	2.2	Grey-black
PM	Pre-evaporator, middle	3.6	Black-grey
PB	Pre-evaporator, bottom	3.2	Black
1T	1 st effect, top	0.9	Light greyish tan
1M	1 st effect, middle	0.8	Light grey
1B	1 st effect, bottom	1.4	Grey-brown
2T	2 nd effect, top	0.4	Grey-brown
2M	2 nd effect, middle	0.4	Grey
2B	2 nd effect, bottom	0.6	Grey
3T	3 rd effect, top	0.5	Dark brown
3M	3 rd effect, middle	1.5	Grey brown flakes
3B	3 rd effect, bottom	1.7	Grey-brown flakes
4T	4 th effect, top	9.3	Off-white, bulky
4M	4 th effect, middle	12.5	Off-white, bulky
4B	4 th effect, bottom	5.6	Grey-brown, bulky

Table 3 shows the results for the four effects and pre-evaporator from one mill. No clear relationship could be determined about deposition along the length of the tubes. Iron deposited at the bottom of the fourth effect in much higher concentration than any other area (3.25% vs usually <0.5%). Silicate was highest at the bottom of the third effect and the top and middle of the fourth effect.

Table 4 shows the composition of the juice from cane variety CP 85-384, the predominant cane variety being harvested at this time in Louisiana. It is evident that high levels of scale-causing compounds enter the factory with the cane juice.

Table 5 shows the average scale composition from the Louisiana growing areas for several key elements.

Table 3. Results of scale analysis by ICP (% of scale) Results corrected for excess of copper*

Sample	Mg	Ca	P	S	Fe	Al	Ni	Cu	SiO ₂	Other‡
PT	2.83	24.86	4.86	0.72	0.55	0.08	0	0.20	0.87	65.03
PM	2.72	17.30	5.44	0.59	0.31	0.10	0	0.20	1.59	71.75
PB	2.88	19.71	7.97	0.64	0.42	0.14	0	0.20	1.43	66.61
1T	5.49	21.19	5.49	0.63	0.83	0.08	0	0.20	9.94	56.15
1M	4.22	26.05	7.05	0.74	0.49	0.08	0	0.20	4.96	56.21
1B	3.72	19.94	6.71	0.63	0.71	0.07	0	0.20	3.98	64.04
2T	1.84	19.07	13.35	0.67	0.59	0.08	<0.01	0.20	2.93	61.27
2M	1.85	27.96	12.70	0.66	0.52	0.06	<0.01	0.20	1.23	54.82
2B	4.26	20.88	9.20	0.82	1.71	0.10	0	0.20	1.60	61.23
3T	1.42	16.44	11.35	0.75	0.48	0.05	<0.01	0.20	3.62	65.69
2M	1.68	19.12	10.03	0.73	0.33	0.04	<0.01	0.20	2.59	65.28
3B	1.78	18.44	9.39	0.73	0.43	0.04	<0.01	0.20	14.48	54.51
4T	2.39	13.53	2.17	0.42	0.38	0.02	<0.01	0.20	15.39	65.50
4M	2.15	10.94	1.97	0.35	0.26	0.02	<0.01	0.20	19.90	64.21
4B	1.76	13.09	3.91	0.47	3.15	0.02	<0.01	0.20	3.79	73.61

* A copper value of 0.20% was assigned to all samples.

‡ Difference between inorganic total and 100%.

Table 4. Composition of CP 85-384 juice.

<u>Analyte</u>	<u>Result (ppm Brix)</u>
Calcium	1833
Magnesium	1120
Potassium	1.19% solids
Chloride	7036
Phosphate	2331
Sulfate	2838
Soluble silicate	64.5
Citric acid	1730
Aconitic acid	5390
Conductivity ash	2.93% solids

Table 5. Average scale composition from Louisiana growing areas.

<u>Growing area</u>	<u>Mg</u>	<u>Ca</u>	<u>P</u>	<u>SiO₂</u>
Bayou Lafourche	0.3	11.1	0.9	27.8
Bayou Teche	1.8	14.8	5.2	13.5
Mississippi River	1.7	15.0	3.4	15.4

The average composition of the scale from the effects is graphed in Figure 1 for calcium, magnesium and silicate, showing some trends in deposition in various effects. For example, calcium was highest in the first two effects; phosphorus peaked in the second and third effects and silicate generally increased across the effects. Weight of scale was highest in the pre-evaporator and fourth effect.

Composition of Scale in Various Effects

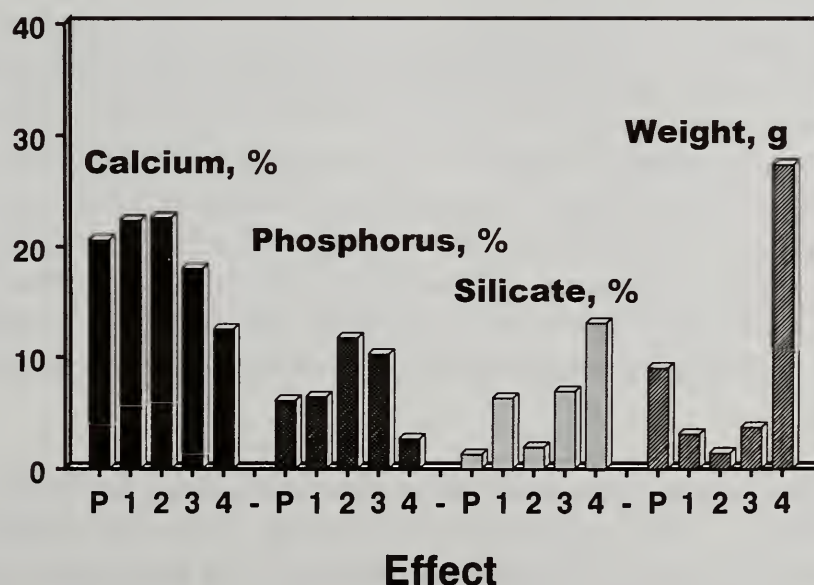
**Figure 1.** Changes in composition of scale in various effects. (Mill in Bayou Teche area.)

Figure 2 shows the average composition of magnesium, calcium, phosphorus and silicate in evaporator scale from the Louisiana growing areas.

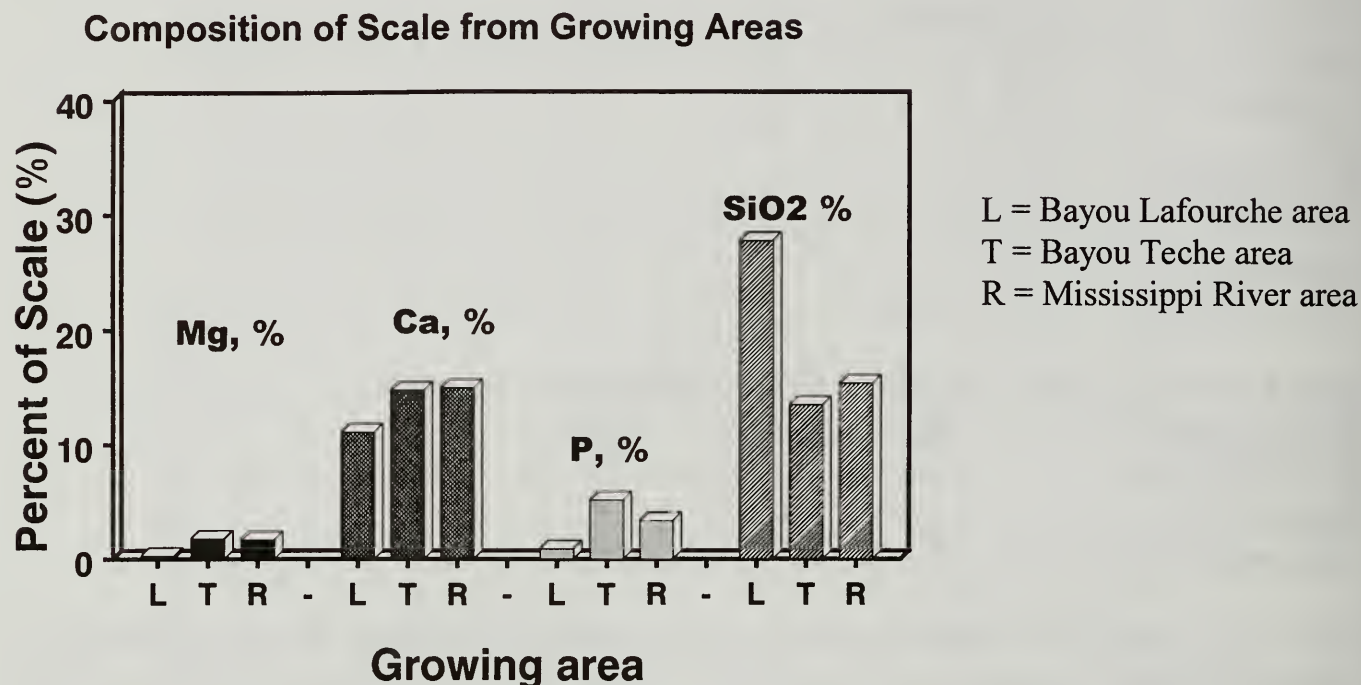


Figure 2. Average composition of scale from the Louisiana growing areas.

CONCLUSIONS

These preliminary results show that the scale problem is extensive in Louisiana and that the scale has a complex composition, not an unexpected result. The main components of the scale in Louisiana are calcium and silica, accounting for 30-50% of the material. The remainder consists of organic material, carbonates and minor metals. Although some of the mills claim they don't have a scale problem, what they actually mean is that they are well adjusted to dealing with the scale at their mill, and it is part of their routine maintenance. However, the cost of cleaning chemicals is expensive, as is their disposal, as well as the downtime or slowed time caused by frequent clean-outs. If antiscalants are used, the cost is increased, but the time between boil-outs can be decreased, so there is a trade-off.

No clear relationships could be determined about deposition along the length of the tubes from different effects (Table 3). Iron deposited at the bottom of the fourth effect in much higher concentration than any other area and silicate appeared higher in the bottom of the third effect and the top and middle of the fourth effect. The scale was very bulky in the fourth effect. The different colors of the scale represent the varying composition. Different rates of deposition will be determined by the solubility of the elements at various syrup concentrations and the organic matrix.

However, overall trends were evident in the amounts of calcium, phosphorus and silicate noted in the various effects (Figure 1) with calcium scale being highest in the first and second effects, phosphorus peaking in the second and third effects and silicate increasing over the effects to peak at the fourth effect.

The three growing areas in Louisiana have similar scale profiles (Figure 2) with somewhat higher silica in the Bayou Lafourche area and phosphorus in the Bayou Teche area.

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GUMS OBTAINED FROM BAGASSE AND FILTER CAKE MUD WITH ALKALINE HYDROGEN PEROXIDE

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ABSTRACT

Techniques developed for isolating hemicellulose gums from corn fiber (Doner, *et al.*, 1998) were adapted to bagasse, the fibrous residue left after cane juice has been extracted from cane plants. Not only are the gums from bagasse interesting in their own right as products with potential pharmaceutical, cosmetic or food applications, they are also examples of some of the type of material that can be stripped out of cane during processing, when lime is added. The procedure, which utilizes sodium hydroxide, calcium hydroxide and hydrogen peroxide, produces two alkaline soluble hemicellulose products, plus a decantate enriched in phenolic acids and cell wall polysaccharide. The major product is Hemicellulose B, an arabinoxylan. Yield of hemicellulose B was in the range of 5-8%, depending on the form the bagasse was in. A second, minor fraction, designated Hemicellulose A, was a glucoarabinoxylan, with a yield of 0.2-3.8%, contained only 25% carbohydrate. Following precipitation and removal of the hemicellulose, a golden yellow alkaline ethanolic decantate remained. The dialyzed and freeze dried decantate had a yield of 1.2% from bagasse and contained about 50% polysaccharide, with a composition typical of cane cell wall polysaccharide. The decantate was enriched in p-hydroxycinnamic acid and other phenolic acids.

Filter cake mud, a by-product of cane sugar processing, was also a source of hemicellulose, with a yield of 2.9%, and of a decantate containing polysaccharide, phenolic acids, and a waxy fraction containing numerous lipid compounds.

INTRODUCTION

Bagasse, the fibrous residue left over after sugarcane stalks are milled to extract sucrose, is the major by-product of sugar processing. Over 900,000,000 tons are produced annually worldwide. Uses for bagasse include energy production by burning (75-80%), combination with molasses and other ingredients for cattle food (10-15%), paper and building materials (1-2%), feedstock for ethanol and

other chemicals (<1%). Bagasse composition is approximately 50% moisture, 48% fiber and lignin and 2% sucrose residue (Paturau, 1982). An ongoing part of the SPRI research mission is to examine new products from cane and beet sugar processing. Gums from fibrous plant material, such as bagasse, may be of interest as potential products, with pharmaceutical, cosmetic or food applications.

Several procedures have been studied for obtaining gums from corn fiber (Doner and Hicks, 1997; Doner, et al., 1998; Hespell, 1998). The procedure developed by Doner, et al., (1998) was adapted to bagasse to obtain bagasse gum. The method uses sodium hydroxide, calcium hydroxide and hydrogen peroxide treatment of the fiber to obtain two alkaline soluble fractions, designated Hemicellulose A and Hemicellulose B. The same procedures were adapted to filter mud, a waste product of sugar processing. Filter mud is composed of precipitated protein and polysaccharide from cane juice, calcium salts, field soil, and fine particles of bagasse.

METHODS

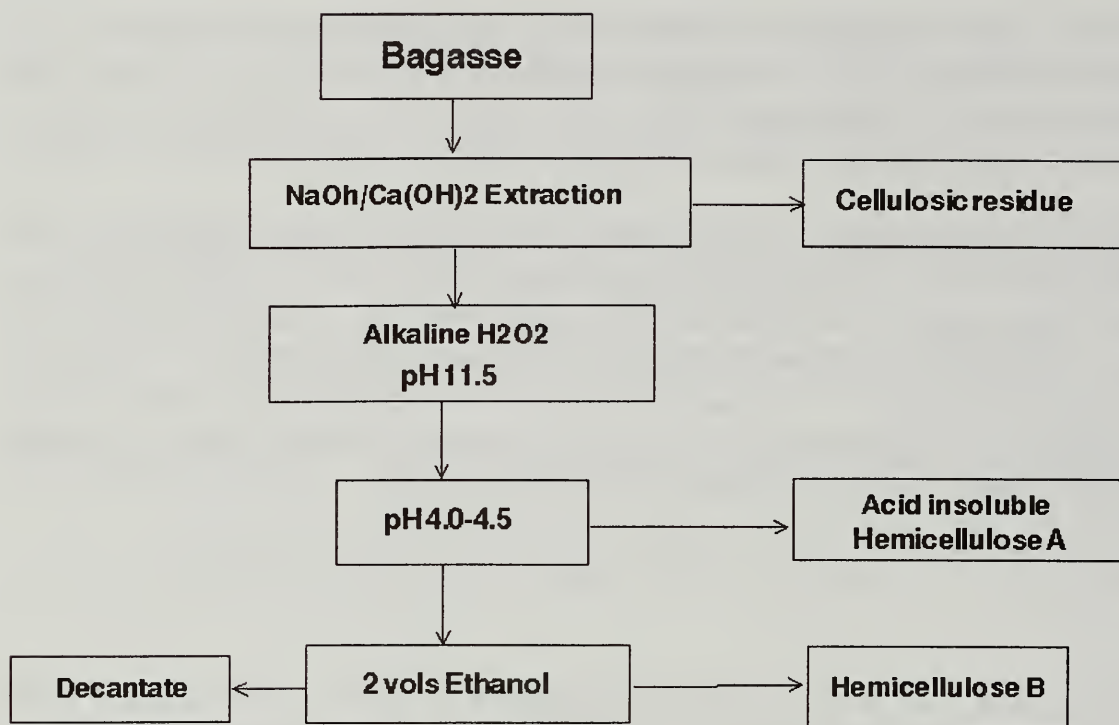
The method of Doner, *et al.*, (1998) for corn fiber was adopted to bagasse. Ten grams of finely ground bagasse was mechanically stirred into 100 ml water, with 0.8 g sodium hydroxide and 0.7 g calcium hydroxide. The mixture was boiled with mechanical stirring for one hour. The unreacted residue was removed by vacuum filtration; residue was washed with 500 ml water. To the 600 ml of extract was added 3 ml 30% hydrogen peroxide. pH was adjusted to 11.5 with 50% NaOH, and the solution stirred for 2 hours at room temperature. Following this period, the solution was acidified to pH 4.0-4.5 by adding concentrated HCl. Upon addition of acid, a small proportion of acid-insoluble hemicellulose (Hemicellulose A) precipitated out. This could be recovered by filtration on a bed of celite filter aid. The remaining gum (Hemicellulose B) was recovered by adding two volumes of 95% ethanol. The decanted ethanol (decantate) was also saved for further study.

Fresh bagasse was obtained from a mill in Louisiana. It was dried overnight in an atmospheric oven at 60°C to stabilize against mold growth. The dried bagasse was then coarsely ground to about 20 mesh in a Wiley mill.

The gums were hydrolyzed with trifluoroacetic acid (TFA) and the sugar composition was determined by GC/MS. Molecular weight of isolated gums was determined by GPC.

During the acidification step to isolate Hemicellulose A from filter mud, a profuse amount of white, waxy material floated to the surface.

The flowchart of the isolation procedure is shown in Figure 1.

**Figure 1.**

Flow chart of procedure for obtaining hemicellulose gums from bagasse.

RESULTS

Bagasse Hemicellulose B

We first examined the hemicellulose from dewaxed rind and derinded bagasse from fresh stalks of cane variety CP 70-321. The average molecular weight of the derinded bagasse (66,580 Da) was about six times higher than that from the rind (10,618 Da), and considerably more heterogenous, as shown by the MW/MN value of 22. The yield from the derinded bagasse (5.0%) was twice that from the rind (2.5%). However, the composition of neutral sugars was similar, except that the rind gum contained 8.8% glucose and the derinded bagasse (pith) contained almost no glucose. Both were arabinoxylans with a ratio of 3:1 arabinose:xylose.

Subsequent studies were conducted on bagasse obtained from a factory in Louisiana. This bagasse is representative of material available for gum isolation; it includes the rind and is composed of a number of cane varieties. Table 1 shows the composition and yield obtained from various preparations. Bagasse Hemicellulose B is a glucoarabinoxylan. The factory bagasse gum had considerably more glucose than the gum from the CP 70-321 variety.

All the samples of Hemicellulose B were light beige and fluffy, giving a clean, pleasant appearance with no odor..

Table 1. Composition of Hemicellulose B from factory bagasse.

Code	Description	% Yield	% CHO	% Neutral sugar composition, normalized					
				Ara	Rha	Xyl	Man	Gal	Glc
B1	Ground bagasse	8.5	84.2	11.2	--	54.7	--	2.0	32.2
B2	Ground bagasse	6.4	>97	15.2	--	58.6	--	1.8	24.3
B4	Ground bagasse, coarse fxn. (0.065")	5.2	>97	13.7	0.8	61.8	--	2.4	21.4
B5	Ground bagasse, fines fxn. (0.0232")	6.6	>97	10.5	--	61.8	--	1.7	26.0
B6	Whole bagasse	5.8	92.3	6.5	--	49.9	--	0.9	42.6
B10	Whole bagasse, 2 x NaOH	4.8	69.4	18.6	--	58.6	--	2.9	19.9

CHO = total carbohydrate content; Ara = arabinose; Rha = rhamnose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose.

Yield is calculated on the basis of the weight of the oven-dried bagasse.

Bagasse Hemicellulose A

The Hemicellulose A was a minor fraction, with the composition shown in Table 2. The neutral sugar composition of Hemicellulose A is very similar to that of Hemicellulose B, but it was insoluble in acid solution. It did not go fully into aqueous solution, and total carbohydrate content was only around 25%. The coarse portion of the bagasse contained more Hemicellulose A than the bagasse fines or the whole bagasse.

Table 2. Composition of Hemicellulose A from factory bagasse.

Code	Description	% Yield	% CHO	% Neutral sugar composition, normalized					
				Ara	Rha	Xyl	Man	Gal	Glc
A1	Ground bagasse	0.2	24.5	13.6	--	53.7	--	4.2	28.6
A4	Ground bagasse, coarse fraction	3.8	27.0	11.9	--	65.4	--	2.5	20.1
A5	Ground bagasse, fines fraction	Trace	No sample was recovered						

Decantate

Following the precipitation and removal of the hemicellulose, a golden yellow alkaline ethanolic decantate remained. The decantates contained phenolic acids, free sugars, glycerol, acids associated with alkaline degradation of carbohydrates, and soluble polysaccharide. The major compound present was p-hydroxy-cinnamic acid. Other phenolic acids were meta-hydroxy-cinnamic acid, and ferulic acid. Sugars included sucrose, glucose and fructose. Alkaline degradation products included lactic, glycolic and glyceric acids.

The dialyzed decantates (from which all compounds with a molecular weight less than 12,000 Da were removed) were light tan, fluffy material with no odor. The yield of dialyzed decantate was about 1.2% on bagasse. The carbohydrate composition of the hydrolyzed decantates is shown in Table 3. The carbohydrate composition is that of typical cane cell wall polysaccharide. The carbohydrate content was on the order of 40-50%; a portion of the decantate was insoluble in water.

Table 3. Composition of dialyzed decantate from factory bagasse alkaline hydrogen peroxide treatment.

Code	Description	% Yield	% CHO	% Neutral sugar composition, normalized					
				Ara	Rha	Xyl	Man	Gal	Glc
D1d	Ground bagasse	1.2	50.9	36.5	2.1	33.3	0.8	15.9	11.4
D2d	Ground bagasse	1.2	41.9	31.8	1.9	34.2	0.8	14.4	16.9

Gum and Other Value-Added Components in Filter Cake

Products extracted from filter cake mud from a Louisiana factory included gums, sterols, waxy alcohols and phenolic and organic acids. An alkaline hydrogen peroxide treatment similar to that for obtaining bagasse gum, yielded 2.9% Hemicellulose B from the filter cake. The appearance of filter cake Hemicellulose B was just as clean and light colored as that from bagasse. The composition of hydrolyzed Hemicellulose B from filter cake is shown in Table 4. The sugar composition is quite different from that of bagasse hemicellulose, with a higher arabinose, rhamnose and galactose content and a lower glucose content.

The freeze-dried decantate from the filter cake was a golden color with a sweet, aromatic odor. The sugar composition of the dialyzed, hydrolyzed decantate is shown in Table 5. Again, there are differences in the carbohydrate composition when compared to bagasse decantate, with a lower xylose content and higher mannose and galactose content. The compounds identified by GC/MS in the decantate prior to dialysis are listed in Table 6.

Table 4. Composition of fractions obtained from filter cake mud from a Louisiana factory.

Code	Description	% Yield	% CHO	% Neutral sugar composition, normalized					
				Ara	Rha	Xyl	Man	Gal	Glc
B7	Hemicellulose B	2.9	94.7	24.1	4.1	46.3	--	11.0	14.6
D7d	Dialyzed decantate	1.3	34.6	33.9	1.9	15.5	5.3	30.0	13.4

CHO = total carbohydrate content; Ara = arabinose; Rha = rhamnose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose.

Table 5. Compounds identified in the decantate from alkaline hydrogen peroxide treatment of filter cake.

Lactic acid
 Glycolic acid
 Glycerol
 2-Hydroxybutanoic acid
 Glyceric acid
 Aconitic acid
 2,4-Dihydroxybutanoic acid
 Fumaric acid
 Aconitic acid
 Tartaric acid
 Citric acid
 Fructose
 Glucose
 p-Hydroxy-cinnamic acid
 Ferulic acid
 Gluconic acid
 Sucrose

Upon addition of acid to precipitate filter cake Hemicellulose A from the filter cake, a profuse amount of white, waxy material floated to the surface. The amount of Hemicellulose A produced was small, and no further characterization of it was done. The waxy material was extracted with boiling ethanol and examined by GC/MS. The composition of the material is shown in Table 6. (Recall that this fraction has already undergone an alkaline hydrolysis to release the fatty materials.) Currently, there is a great deal of interest in plant sterols and high molecular weight fatty alcohols for their beneficial

physiological properties. It is also possible to extract the waxy material from filter cake with solvents, such as petroleum ether or boiling ethanol and obtain a similar composition to that in Table 6.

Table 6. Compounds identified in the waxy fraction of filter cake mud.

Compound	Description
Lactic acid	Alkaline degradation product
Glycerol	Backbone for fatty acid esters
Aconitic acid	Sugarcane acid
Quinic acid	Sugarcane acid
p-Hydroxy-cinnamic acid	Phenolic acid, a major component
Sucrose	Extraneous, entrained with material
Palmitic acid	C-16 straight chain fatty acid, a major component
Linoleic acid	C-18 fatty acid (9,12,15-octadecatrienoic acid), a major
Oleic acid	C-18 fatty acid (9-octadecenoic acid), minor component
Stearic acid	C-18 straight chain fatty acid, minor component
Hexacosanol	C-26 straight chain waxy alcohol
Octacosanol	C-28 straight chain waxy alcohol, the major component
C29-OH	C-29 straight chain waxy alcohol
C31-OH	C-31 straight chain waxy alcohol
C32-OH	C-32 straight chain waxy alcohol
Stigmasterol	Plant sterol
beta-Sitosterol	Plant sterol
Campesterol	Plant sterol

CONCLUSION

Hemicellulose B, obtained in about 6.5% yield from bagasse by alkaline hydrogen peroxide treatment is an arabinoxylan with potentially interesting properties. Products of interest obtained from alkaline hydrogen peroxide treatment of filter cake include phenolic acids and plant sterols.

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A NOTE ON HEMICELLULOSE GUMS FROM BEET PULP EXTRACTED WITH ALKALINE HYDROGEN PEROXIDE

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INTRODUCTION

Studies at SPRI on potential added-value products have focused on gums obtained from bagasse (Godshall, *et al.*, 2002) and on beet pulp. Beet pulp is the major by-product of the beet sugar industry, and much work has been done to elucidate its properties and composition. Although its main use today is in animal feed, a small market exists for edible beet fiber. Thibault has been in the forefront of characterizing beet pulp for added value (Thibault and Bonnin, 2000) and has been especially interested in its ferulic acid content (Micard, *et al.*, 1997; Colquhoun, *et al.*, 1994; Ralet, *et al.*, 1994; Micard, *et al.*, 1994; Bertin, *et al.*, 1988). Sun and Hughes (1998) conducted fractional alkaline extraction of sugar beet pulp.

Studies by Godshall on sugarcane bagasse, using the alkaline hydrogen peroxide procedure of Doner to extract hemicellulose (Doner, *et al.*, 1998.), showed a yield of about 6.5% from bagasse of a highly purified arabinoxylan (Godshall, *et al.*, 2002). The same procedure was used to extract hemicellulose from industrial sugar beet pulp.

MATERIALS AND METHODS

The procedure of Doner, *et al.*, (1998) was used to extract the hemicellulose. Samples of beet pulp were obtained from industrial sugarbeet operations and used as received, without further washing, grinding or other treatment. Fifty grams of beet pulp was mechanically stirred into 500 ml water, with 4.0 g NaOH and 3.5 g Ca(OH)₂, and boiled for one hour, with stirring. The undissolved residue was removed by vacuum filtration. To the extract was added 5 g 30% hydrogen peroxide, and the pH was adjusted to 11.5 with 50% NaOH. The solution was then stirred for two hours, after which the pH was adjusted to 4.0-4.5 with concentrated HCl. (No precipitate formed.) Two volumes of 95% ethanol were added, and again no precipitate formed, so the ethanol was evaporated under rotary vacuum to about 200 ml and the solution dialyzed overnight and freeze dried.

The sugar composition was determined by GC/MS following hydrolysis by trichloroacetic acid. Molecular weight was determined by gel permeation chromatography (GPC).

RESULTS AND DISCUSSION

Unlike corn fiber and cane bagasse, the beet pulp did not produce precipitates of Hemicellulose A and Hemicellulose B. Upon acidification of the alkaline peroxide treated extract, both corn fiber and cane bagasse precipitated an acid-insoluble Hemicellulose A fraction, but the beet fiber extract did not. Upon adding ethanol to approximately 65% concentration, the beet pulp did not produce a precipitate of Hemicellulose B, as did corn fiber and cane bagasse. This would indicate that the extracted hemicellulosic material was of a low molecular weight, probably in the range of 10,000 to 12,000 Daltons.

The freeze dried extract was a fluffy, pale beige to off-white material, with a clean, pleasant appearance. This material was designated Hemicellulose B.

The composition of several examples of Hemicellulose B obtained from several samples of beet pulp is shown in Table 1. The yields were variable, ranging from 10% to 54%, and may reflect the way the beet pulp was prepared prior to our receiving it. The analyses showed that material was almost all carbohydrate, with a neutral sugar composition indicating it is an arabinogalactan, consisting mostly of arabinose.

Table 1. Composition of Hemicellulose B from beet pulp.

Code	Yield	Composition	GPC	Total CHO
3-1	10%	Arabinose:galactose (4:1)	MW = 11,132 MW/MN = 1.74	>97%
4-1	51%	ara:gal:glc:rha (10:1:1:1)	MW = 7,239 MW/MN = 4.03	>97%
5-1	54%	ara:gal:glc:rha (12:1:1:1)	MW = 7,425 MW/MN = 2.89	85.5%
8	18%	not determined	not determined	>97%
9	18.5%	not determined	not determined	>97%

CHO = carbohydrate

ara = arabinose; gal = galactose; glc = glucose; rha = rhamnose

The GPC results confirmed that the isolated hemicellulose was of a low molecular weight. The composition is mostly arabinose (75-80%) with up to 25% of galactose, and minor amounts of glucose and rhamnose. GPC showed a single peak, with only a small amount of trailing on either end, which is reflected in the MW/MN of 1.74 to 4.03, a measure of homogeneity/polydispersity.

In the work reported by Sun and Hughes (1998), 10% KOH, 24% KOH, 7.5% or 17.5% NaOH was reacted with depectinated sugar beet pulp, at 15° C for 2 hr or 16 hr. They obtained about 10% yields, but the composition, molecular weight and polydispersity of material obtained varied somewhat, depending on the strength of the alkali used and whether sample was delignified or not. They observed low molecular weight products, in the range of 6,000 to 10,000 Daltons, and speculated that the high concentration of alkalinity may have contributed to some destruction of the hemicellulose. In our experiments, the concentration (0.8%) of NaOH was much lower, but samples were subjected to boiling for 2 hours. It is likely that the use of alkaline hydrogen peroxide increased the yield of hemicellulose due to its delignification activity, as reported by Doner, *et al.*, (1998) for corn fiber. It also contributed to the production of a whiter, cleaner appearing product.

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IDENTIFICATION OF ORGANIC COMPOUNDS IN VINASSE

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ABSTRACT

The transformation of vinasse, a high-volume by product of alcohol production, has been a challenge to the alco-chemical industry. The major organic compounds of vinasse were identified and characterized, using methods of liquid-liquid extraction, dialysis and combined analysis by gas chromatography-mass spectrometry (GC-MS). Of the 15 organic compounds found in the samples of vinasse supplied by Sucromiles S.A. (Palmira, Valle, Colombia), the major components were glycerol (2.7%), aconitic acid (1.8%), sorbitol (1.4%), lactic acid (1.3%), quinic acid (0.7%), fructose (0.5%) and glucose (0.3%). Other compounds such as 1, 3-butanediol, furfuryl alcohol, benzaldehyde and 3-methoxyacetophenone were also characterized, using solid-phase microextraction (SPME) and GC-MS.

INTRODUCTION

The production of alcohol in Colombia employs cane molasses as a substrate and brewers yeast, *Saccharomyces cerevisiae*, as the biological catalyst (Bravo and Giraldo, 1996). Vinasse is a high-volume byproduct, generated in a ratio of 14 liters of vinasse per liter of alcohol produced (Lyons, *et al.*, 1995). Vinasse is characterized by an acid pH (4.2-4.6) and high organic matter content, dissolved and in suspension. It also has an appreciable amount of inorganic salts based on sulfates and phosphates of calcium, potassium, sodium and magnesium (Pontificia Universidad Javeriana, 1995).

The transformation of vinasse has been a challenge for the alco-chemical industry. In the last few years applications of fertirrigation, compost production, recovery of soils and animal feed have been evaluated and developed (Sarria, 1995), as well as biodegradation via processes of anaerobic and aerobic fermentation and as a cultural medium for producing single-cell protein (Bravo and Giraldo, 1996).

Dowd and collaborators (1994) report the presence of lactic acid, glycerol, ethanol and acetic acid in vinasse. These researchers used techniques of gas chromatography, high performance liquid chromatography (HPLC) and mass spectrometry to identify these organic compounds of vinasse.

METHODOLOGY

Three samples of vinasse from Sucromiles S.A. were collected during the months of April, August and November of 1998. The last sample was concentrated at reduced pressure under laboratory conditions (0.234-0.267 atm, 88-90°C) to 63% dry matter. In the samples of vinasse from April and August 1998, the organic compounds were separated in accordance with the route of liquid-liquid extraction reported for raw sugars (Giraldo, 1995).

The vinasse samples were partitioned with chloroform, followed by a second extraction of the aqueous fraction with ethyl acetate. The organic fraction was submitted to retroextraction with a 1:9 mixture of ethyl ether:ethanol (see Figure 1).

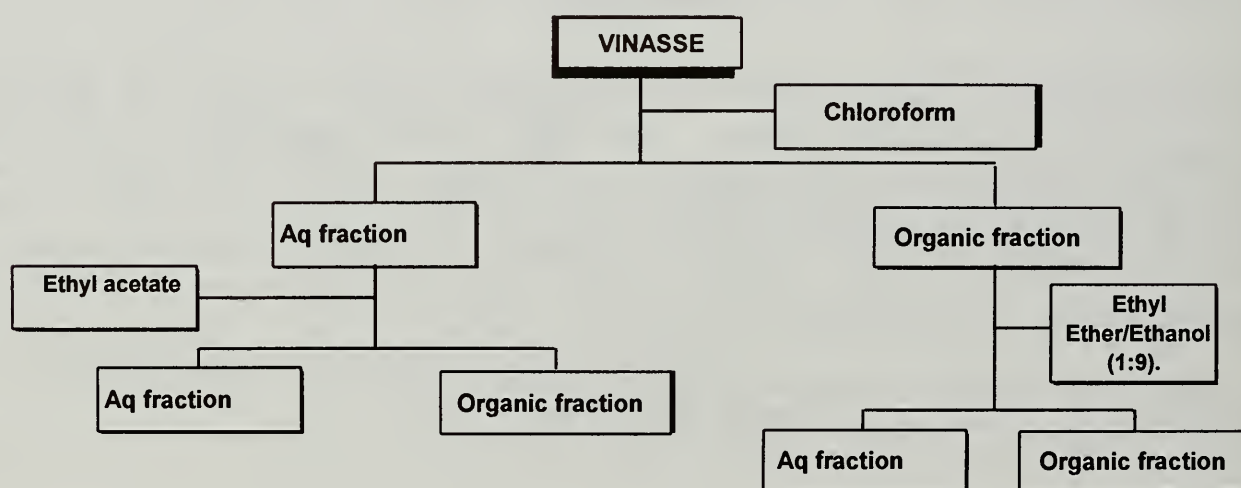


Figure 1. Liquid-liquid extraction of vinasse.

The resulting organic fractions were derivatized with trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) to obtain the trimethylsilyl ethers. The sample was dissolved in pyridine. The mixture was heated at 85°C to ensure complete derivatization. The organic compounds were identified and quantified by gas chromatography and mass spectrometry (GC-MS), using xylitol as the internal standard.

The sample of concentrated vinasse was derived in pyridine with TMCS and HMDS and analyzed by GC-MS. The sample was submitted to dialysis to obtain and quantify the compounds of high molecular weight (nondialyzable fraction). Regenerated cellulose with a nominal molecular weight of 12,000 Daltons was used for this purpose.

The volatile compounds present in the vinasse were determined using solid-phase microextraction (SPME), followed by GC-MS analysis.

RESULTS AND DISCUSSION

The highest percentage (61.4%) of compounds extracted from the vinasse (see Diagram 1) was obtained via extraction with ethyl acetate, where the principal constituent was glycerol (30.0%). Fractions obtained with other extractants, such as ethyl ether and chloroform, in the liquid-liquid extraction of vinasse, were characterized as phenolic compounds.

Dialysis of concentrated vinasse made it possible to separate compounds with a molecular weight of 12,000-14,000 daltons. The nondialyzable fraction of vinasse represented 4.9% of solids, which corresponded to mixtures of compounds of very high molecular weight such as polysaccharides (3.82%) and colorants.

In the concentrated vinasse the descending order of concentration for the following compounds, expressed in percentages of solids is shown in Table 1: glycerol (0.70%), aconitic acid (1.80%), sorbitol (1.40%), lactic acid (1.30%), quinic acid (0.70%), fructose (0.50%) and glucose (0.30%).

With the SPME technique, volatile compounds such as 2,3-butanediol, furfural alcohol, benzaldehyde and 3-methoxyacetophenone were identified.

Table 1. Composition of concentrated vinasse (64.8 °Brix).

COMPOUND	COMPOSITION (% solids)
2,3-butanediol	0.01
2-methyl-1,3-butanediol	0.20
Glycerol	2.70
Sorbitol	1.40
Lactic acid	1.30
Succinic acid	0.07
Malic acid	0.23
Aspartic acid	0.05
Aconitic acid	1.80
Citric acid	0.80
Quinic acid	0.70
Fructose	0.50
Glucose	0.30
Sucrose	0.20
Trehalose	0.30

CONCLUSIONS

- Based on the samples of vinasse from Sucromiles S.A., a wide range of organic compounds were found: alcohols, aldehydes, ketones, esters, acids and phenolic compounds.
- Analysis of concentrated vinasse revealed the following principal compounds based on their composition: glycerol, lactic acid and sorbitol.
- Given the commercial importance of compounds such as glycerol, lactic acid, sorbitol and 3-methoxyacetophenone, technical-economic evaluations for studying the best commercial route of extraction and exploitation of these and other compounds of vinasse should prove fruitful.

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THE ROLE OF ENZYMES IN POLYSACCHARIDE DEGRADATION IN CANE SUGAR PROCESSING

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ABSTRACT

Enzymes have proven useful in raw cane sugar manufacture to control polysaccharides that affect processing and quality; namely amylase, used to control starch, and dextranase, used to control dextran. A third important polysaccharide of interest in cane sugar processing is ISP, indigenous sugarcane polysaccharide, a suite of soluble cell wall polysaccharides that is expressed into raw juice on the order of 0.5% on solids. Depending on the condition of the cane at harvesting, especially if it is harvested green, the amount can be higher. ISP contributes most abundantly to the concentration of total polysaccharide found in cane process streams and can be blamed for several problems. Because it is associated with phenolics, it contributes to a great deal of color in the juice, and has a tendency to transfer to the sugar crystal; it has been implicated in acid beverage floc formation and turbidity in refined sugar; and contributes to the high level of viscosity in cane molasses.

The ability of several commercial enzymes to break down ISP was tested in mixed cane juice. Unlike starch and dextran, there are no specific enzymes available for ISP degradation, and this study reports preliminary efforts in that direction. Three enzymes were found to degrade ISP -- Midland SucroDex C Super, Novo Celluclast and Novo Viscozyme. Viscozyme degraded sucrose and Celluclast was inactivated at 75° C.

INTRODUCTION

Polysaccharides are long-chain carbohydrate polymers that have important physiological effects in plants and animals. In sugarcane processing, several polysaccharides are of interest because they have significant effects on processing and quality of sugar. These include the well-known effects of starch, a natural component of the sugarcane plant, which is expressed into cane juice, and dextran, a by-product of infection with the bacteria, *Leuconostoc mesenteroides*. These two polysaccharides cause increases in viscosity, difficulty in purging, carry over into sugar crystals, and, in the case of dextran, represent sucrose loss due to microbial action.

A great deal of research has been done to understand the nature of polysaccharides in sugarcane and to find ways to minimize their effects (for example, Imrie and Tilbury, 1972; Hidi, *et al.*, 1976; Coll, *et al.*, 1978; Godshall, *et al.*, 1990; Godshall, *et al.*, 1994; Clarke, *et al.*, 1997; Edye, *et al.*, 1997; Johnson, 1989).

Enzymes have proven useful in raw cane sugar manufacture to control polysaccharides that affect processing and quality; namely amylase, used to control starch, and dextranase, used to control dextran. A third important polysaccharide of interest in cane sugar processing is ISP, a suite of indigenous sugarcane polysaccharides, soluble cell wall polysaccharides that are expressed into raw juice on the order of 0.5% on solids (5000 ppm). Depending on the condition of the cane at harvesting, especially if it is harvested green, this amount can be much higher (Godshall, *et al.*, 2000). ISP contributes most abundantly to the concentration of total polysaccharide found in cane process streams. Because it is associated with phenolics, (Godshall, *et al.*, 2000) it contributes to a great deal of the color in juice, and has a tendency to transfer to the sugar crystal; it has been implicated in acid beverage floc formation and turbidity in refined sugar; and contributes to the high level of viscosity in cane molasses. There are indication that ISP may also be implicated in slowed boiling times and poor crystallization.

MATERIALS AND METHODS

Enzymes Examined

Novo Viscozyme L. A multi-component enzyme containing a wide range of carbohydrases, including arabinase, cellulase, beta-glucanase, hemicellulase and xylanase. It is used to break down plant cell walls for protoplast research.

Midland SucroDex C Super. A multi-component enzyme containing dextranase, amylase, cellulase, beta-glucanase and xylanase.

Novo Celluclast. A cellulase enzyme that breaks cellulose down to glucose.

Novo Termamyl 120L. Alpha-amylase, breaks down starch; heat stable.

Novo BAN 240L. Alpha-amylase, breaks down starch.

Novo AMG 300L: Glucoamylase.

Solway L4000: Dextranase.

Experimental

Enzymes were added to mixed juice at 500 ppm (75mg/150 ml juice). The samples were treated in a gyratory water bath for two different times (1 and 4 hr) and at two temperatures (50° C and 75° C). All analyses were done in duplicate. Mixed juice was obtained from a nearby mill. The juice samples had brix and pH of 11.7 Bx, pH 5.50 and 13.5 Bx, and pH 5.30, respectively.

Control No. 1 (untreated) consisted of the original juice samples, prior to any treatment. Control No. 2 (heated) consisted of the original juice samples, subjected to the time and temperature regimes listed, but without enzyme treatment.

Analyses performed included: Total polysaccharides, starch, dextran, sucrose, glucose, and fructose (oligosaccharides noted as reported). ISP was calculated as the difference between total polysaccharide minus the sum of starch and dextran.

A medium was developed, ISP medium, that provided ISP as the sole source of carbon.

RESULTS AND DISCUSSION

Of the seven enzymes studied, three gave significant results: Novo Viscozyme L., Midland SucroDex C Super, and Novo Celluclast. Heat alone removed a significant amount of ISP, which was reduced by as much as 30.6% in the final treatment of 75° C for 4 hr (Figure 1).

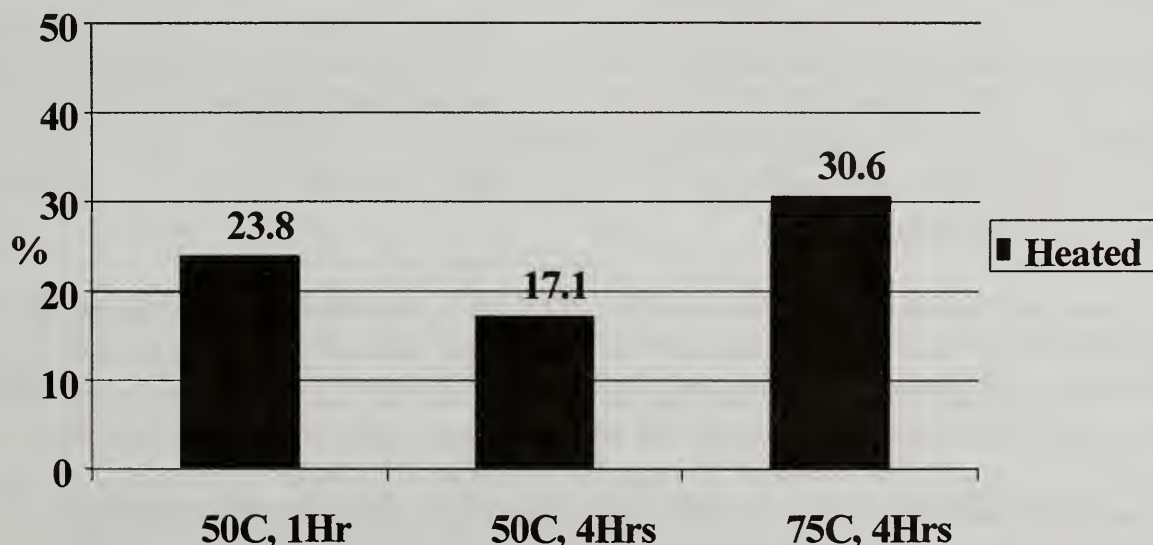


Figure 1. Removal of ISP by heat.

Table 1 shows the results for treatment at 50° C for 1 hr. It is noted that the results are complicated by what appears to be the increased formation of dextran in all the heated samples. Heat treatment alone reduced ISP by 23.8%. ISP was additionally reduced by Viscozyme and SucroDex by 46.8% and 12.2%, respectively (compared to heat treated results). Compared to the unheated control, Viscozyme decreased ISP by 59.4% and SucroDex reduced ISP by 33.0%.

Viscozyme, however, also reduced the sucrose from 82.4% to 61%, indicating significant sucrose destruction. The chromatogram showed the production of a large quantity of an oligosaccharide peak, representing about 17% of the solids. The destruction of sucrose resulted in only a relatively small increase in glucose, from 2.56% to 3.94%, and no increase in fructose, indicating that the sucrose destroying activity may not have been caused by invertase included in the enzyme mix, but rather by a synthetic (transferase) function of one of the enzymes in the mix. This may warrant further study in the future. It does, of course, preclude Viscozyme as a promising enzyme mixture for use in sugar processing.

Most of the enzymes also significantly reduced the starch content: Viscozyme (49.5%), SucroDex (36.9%), Celluclast (38.0%), Termamyl (16.0%), and glucoamylase (68.6%). It is interesting to note that the Termamyl enzyme was the least effective. The glucoamylase, while reducing starch, also seemed to cause an unexplained increase in ISP.

Table 2 shows results for treatment at 75° C for 1 hr. There is essentially no improvement in enzyme activity with regard to removal of ISP for most of the enzymes, with the exception of Celluclast, which showed a 19.4% reduction in ISP compared to the heat control. The large amount of starch reduction by heat treatment alone is felt to be due to the native starch degrading enzyme in the cane juice. We have seen this many times before, and in fact, usually boil cane juice as soon as possible after collecting it, to prevent this from happening. There is still dextran formation going on under these conditions. Viscozyme was again shown to reduce sucrose content.

Table 3 shows results for treatment at 50°C for 4 hrs. Viscozyme again showed a large decrease in sucrose concentration (from 79.8% to 47.3%) with an increased oligosaccharide content of 20-25% solids, along with a 34.0% decrease in ISP compared to the heat control. SucroDex decreased ISP by 7% and Celluclast decreased it by 16%. Heat alone decreased ISP by 17%. Dextran again increased in all treated samples.

Table 4 shows the results for treatment at 75°C for 4 hrs. As with the results shown in Table 2, Viscozyme had less effect on sucrose concentration and made little oligosaccharide, as well as having no effect on ISP, indicating that this enzyme was inactivated at 75°C. Compared to the heat-treated control, SucroDex removed 17.7% of the ISP and Celluclast removed 25.1% of the ISP.

Figure 2 summarizes the removal of ISP by heat, Viscozyme, SucroDex and Celluclast.

Table 1. Enzyme treatment of mixed juice at 50°C for 1 hour

Enzyme/ Treatment	TPS	Starch	Dextran	ISP	Sucrose %Solids	Glucose %Solids	Fructose %Solids
Untreated	6073	1055	1062	3956	82.40	2.58	2.67
Heat alone	5945	993	2702	3016	82.75	2.56	2.58
Viscozyme	4792	533	2653	1606	60.94	3.94	2.72
Sucrodex	5781	666	2466	2649	81.51	2.81	2.76
Celluclast	5901	654	2216	3031	82.78	2.86	2.82
Termamyl	6531	886	2691	2954	82.82	2.50	2.66
Alpha- amylase	6531	1050	2804	2677	82.09	2.59	2.61
Gluco- amylase	6695	331	1822	4542	78.89	4.32	4.28
Dextranase	6541	1117	2409	3015	78.71	3.64	4.00

TPS = Total polysaccharides

ISP = Indigenous sugarcane polysaccharides

Table 2: Enzyme treatment of mixed juice at 75°C for 1 hour

Enzyme/ Treatment	TPS	Starch	Dextran	ISP	Sucrose %Solids	Glucose %Solids	Fructose %Solids
Untreated	6073	1055	1062	3956	82.40	2.58	2.67
Heat alone	6305	360	2046	3899	82.15	2.65	2.56
Viscozyme	6033	322	1863	3848	80.25	3.49	2.67
SucroDex	5715	319	1530	3866	82.25	2.75	2.85
Celluclast	5429	366	1922	3141	82.74	2.78	2.31
Termamyl	5899	264	2419	3216	83.14	2.79	2.65
Alpha- amylase	6185	391	2269	3525	81.11	2.70	2.72
Gluco- amylase	6295	267	2415	3613	81.54	2.78	2.86
Dextranase	6195	312	2300	3583	81.38	2.74	2.72

Table 3: Enzyme treatment of mixed juice at 50°C for 4 hours

Enzyme/ Treatment	TPS	Starch	Dextran	ISP	Sucrose %Solids	Glucose %Solids	Fructose %Solids
Untreated	7343	1284	2072	3987	79.78	4.12	5.09
Heat alone	6828	1141	2377	3310	79.93	3.92	3.86
Viscozyme	4750	216	2352	2182	47.27	13.68	4.56
SucroDex	6425	822	2524	3079	79.99	3.76	3.65
Celluclast	6285	1131	2374	2781	78.89	3.59	3.50
Termamyl	6145	762	2100	3283	78.15	4.03	3.97
Alpha- amylase	6472	899	2150	3423	78.08	3.85	3.83
Gluco- amylase	6491	580	2287	3624	77.27	5.04	4.71
Dextranase	6847	1045	2212	3590	78.14	3.98	3.92

Table 4: Enzyme treatment of mixed juice at 75°C for 4 hours

Enzyme/ Treatment	TPS	Starch	Dextran	ISP	Sucrose %Solids	Glucose %Solids	Fructose %Solids
Untreated	7343	1284	2072	3987	81.64	2.58	3.07
Heat alone	6480	552	3162	2766	80.67	2.86	3.22
Viscozyme	6917	467	3400	3050	80.05	3.73	3.41
SucroDex	5918	404	3238	2276	81.38	2.93	3.24
Celluclast	5751	429	3250	2072	81.46	2.88	3.12
Termamyl	5508	211	2128	3169	79.72	2.81	2.94
Alpha- amylase	5492	402	1303	3787	79.13	2.94	3.24
Gluco- amylase	6382	406	2475	3501	79.79	3.21	3.31
Dextranase	6627	341	1583	4703	79.03	3.00	3.38

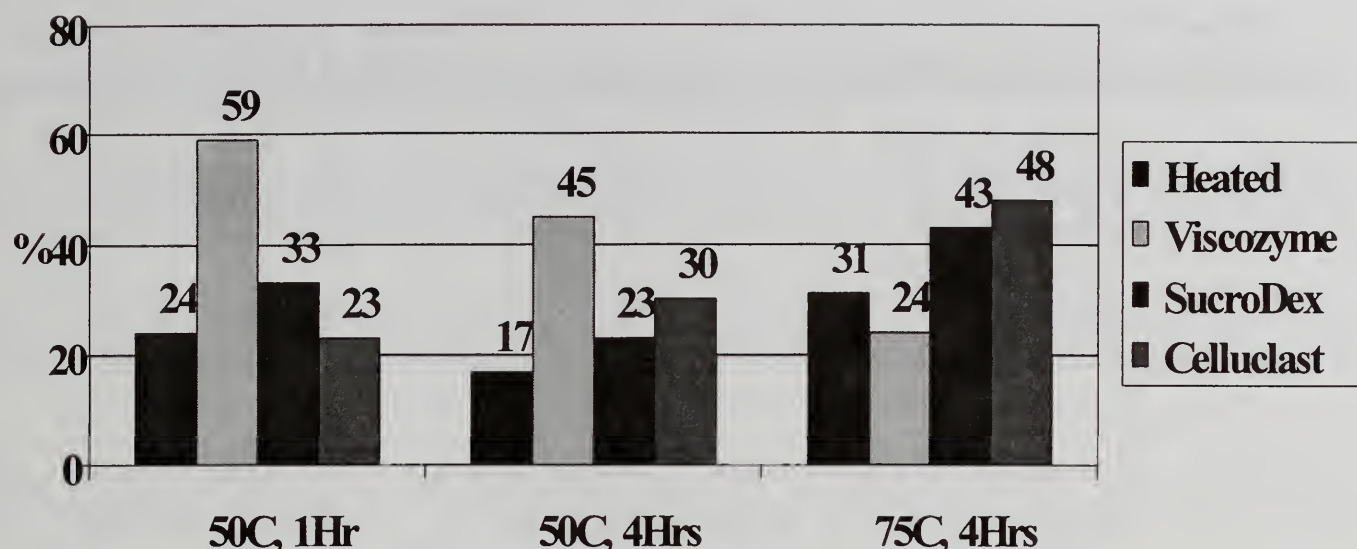


Figure 2. Proportion (%) of ISP removed from mixed juice by various treatments.

Combinations of Enzymes

Celluclast and SucroDex appeared to significantly reduce ISP, without affecting sucrose (purity) or invert content. It was therefore decided to test a combination of these two enzymes at both 50°C and 75°C for 1 hour at both the unadjusted, natural pH of the juice (5.38) and at pH 6.70 to simulate the pH of clarified juice. Enzyme concentration was 500 ppm each. Results are shown in Table 5. To summarize, there appeared to be no adverse effect on sucrose (purity) or invert (no increase) in any of the treatments with this combination of enzymes. The removal of ISP and starch is summarized here:

% ISP and starch removed with a combination of SucroDex and Celluclast

Treatment	% ISP removed	% Starch removed
50°C, pH 5.38	22.5	8.0
75°C, pH 5.38	4.1	23.5
50°C, pH 6.70	24.1	29.2
75°C, pH 6.70	11.9	32.8

The results indicate a drop-off in the ISP removing activity at the higher temperature, but at 50°C, the higher pH does not have an adverse effect. The literature on Celluclast states its optimal activity occurs at pH 5.0 and 60°C, with reduction in activity at >65°C and pH 7.0. The results could indicate that the majority of the ISP removal was done by the Celluclast enzyme under these conditions. Starch was also removed to a significant extent, probably by the amylase activity of the SucroDex.

Table 5. Treatment of mixed juice with a combination of Celluclast and Sucrodex enzymes at two temperatures and two pH levels.

Enzyme Treatment	TPS	Starch	Dextran	ISP	Sucrose %Solids	Glucose %Solids	Fructose %Solids
1	7274	2068	1514	3692	85.6	2.43	2.36
2	6549	1903	1786	2860	86.0	2.22	2.18
3	6748	1326	1849	3573	85.7	2.22	2.23
4	6737	1014	2297	3426	85.7	2.28	2.13
5	7282	1751	1419	4112	80.0	2.08	2.00
6	5984	1240	1625	3119	81.1	1.99	1.95
7	5956	814	1467	3675	80.0	1.98	1.96
8	5362	547	1576	3239	80.5	2.03	1.88

Sample#	Enzyme	Temp	pH
1	-	50	5.38 (unadjusted)
2	+	50	5.38 “
3	-	75	5.38 “
4	+	75	5.38 “
5	-	50	6.70 (adjusted)
6	+	50	6.70 “
7	-	75	6.70 “
8	+	75	6.70 “

Isolation of Microorganisms That Can Utilize ISP as the Sole Source of Carbon

A medium was developed, ISP medium, that provided ISP as the sole source of carbon. Swab culture samples from various locations in a raw sugar mill (choppers, mills, bagasse, etc.) were taken and inoculated onto the ISP medium. After 5 days incubation at 25° C, twenty (20) individual colonies were selected and again placed on ISP medium for ability to produce acid from the ISP carbon source. Although several colonies were able to grow on the media, non were able to ferment ISP with acid production. Further work is required to determine if the non-acid producing bacteria were degrading ISP successfully.

CONCLUSIONS

- ▶ Two enzymes, Novo Celluclast and Midland SucroDex C Super removed significant quantities of ISP in mixed juice. The ISP removing activity of Celluclast was superior to that of SucroDex, but Celluclast activity dropped off at 75°C.
- ▶ Viscozyme, an interesting enzyme with the ability to significantly remove ISP, destroyed sucrose and made oligosaccharides at 50°C. It was inactivated at 75°C.
- ▶ None of the enzymes of interest (Celluclast, SucroDex C, and Viscozyme) appeared to have a significant effect on dextran, which increased in all samples. The conditions of incubation were probably ideal for the growth of any *Leuconostoc* bacteria that were present.
- ▶ Heat alone removed a moderate amount of heat and starch. There may be natural ISP-degrading enzymes in cane juice, as there is natural amylase in cane juice.

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DEXTRAN ANALYSIS WITH POLARIMETRIC, IMMUNOLOGICAL, ROBERTS' AND HAZE METHODS

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INTRODUCTION

Measuring dextran in sugar-containing streams has always been a problem of detecting a small amount of a specific, against the background of a large excess of other, carbohydrates. Most methods have focused on reducing the background by selectively removing dextran by alcohol precipitation. Instrumental methods, to date either have not been selective enough or rapid enough for dextran determinations to be of value as a process control aid to the sugar manufacturer.

Two recent procedures, viz. the Midland SucroTestTM and the Optical Activity Ltd. DASA methods were reported ¹⁻³ to hold promise. Comparison with two established procedures, viz. Roberts'⁴ and Haze⁵ has been undertaken in collaboration between ASI and SPRI. Twenty raw sugar samples were chosen, and three samples of fresh raw juice prepared by milling billeted cane at ASI. Each of the three juice samples was analyzed fresh within ½ hour after milling, and again after up to 24 hours of standing at ambient temperature, in order to monitor the rate of dextran generation as well as to widen the dextran range. The DASA and SucroTestTM procedures were done simultaneously at ASI as the juice sample was withdrawn, while the Roberts' analysis was done at S.P.R.I. after the juice was kept frozen for a week or so. Left-over solutions (juices and sugar liquors) after sample preparation for DASA analysis (filtration and dextranase treatment) were quickly frozen and analyzed again later (Roberts' at S.P.R.I. and SucroTestTM).

Midland SucroTestTM immunological (antibody)¹ assay.

The assay is based upon a linear increase in turbidity with dextran concentration over a set time period. This is determined by adding a fixed volume (10µl) of a solution of the sample to be analyzed to a fixed volume of antibody (1.0 or 2.0 ml), determining the change in NTU reading after one minute and comparing it with the change obtained with a standard dextran solution. The assay is linear for dextran concentrations under these conditions to 1000 ppm on juice. For levels

above 1000 ppm, dilution of the sample is recommended. The dextran-antibody reaction is independent of the size of the dextran, but the formation of turbidity is not. The assay was found less sensitive to dextran with sizes of 40,000 MW or smaller, and no detectable turbidity is formed with dextran of 10,000 MW or less. The Midland SucroTestTM Procedure was performed at ASI.

DASA – Optical Activity Ltd. Polarimetric method^{2,3}.

Change of the optical rotation of a juice sample prior to and after treatment with dextranase is related to dextran concentration. The juice sample is filtered under pressure with DE which is added to facilitate filtration. Pol (deg Z) is read with an 880 nm polarimeter, and again after the treatment with a proprietary dextranase immobilized on a strip of filter paper. Software incorporated in the OA Ltd. SacchAAr 880 polarimeter directly displays the dextran concentration in ppm on juice. The raw sugar samples were diluted 1:2 (33 Bx) with DI water and filtered without diatomaceous earth (DE), as the filtration was rapid and turbidity sufficiently low for the 880 nm polarimeter to give stable readings. A SacchAAr 880 polarimeter with the dextran software was leased from Index Instruments, Inc. (Kissimmee, FL), the U.S. distributor, and used at ASI.

Roberts' Copper Method⁴.

After 80% EtOH precipitation, filtration and washing with water, the precipitate is redissolved in hot water and an alkaline Cu(II) reagent is used to selectively precipitate dextran that is then filtered out and washed. The precipitate is then again redissolved in the presence of phenol-H₂SO₄ reagent that hydrolyses dextran and produces by reaction with glucose a coloration proportional to dextran concentration. The method is reported to be sensitive to dextran with a MW > 10,000 and applicable to raw sugar and raw juice.

Modified Alcohol Haze Method⁵.

Made official by ICUMSA in 1995, it is reported applicable to raw sugar. In the dissolved sample starch is destroyed by incubation with amylase and proteins are removed by precipitation with TCA. The dextran is then detected and quantified with a spectrophotometer at 720 nm after addition of EtOH. Pharmacia T110 or T500 standard dextrans are used to produce a calibration curve.

RESULTS

Dextran analysis with the four methods on 20 raw sugar samples from the SPRI raw sugar library is given in Table I. The SucroTest and DASA procedures were done at ASI while the Roberts' and Haze methods were done at SPRI. With the exception of the polarimetric DASA that was found erratic and did not correlate with the other methods, all others display some degree of correlation, the best being between the Haze and SucroTestTM procedures with a R² of 0.8722 (Figure 1). To study in more detail the characteristics of the DASA procedure, the raw sugar liquors after filtration and after the DASA dextranase treatment were also analyzed with the Roberts' and SucroTestTM procedures. The results, again in ppm dextran on sugar are shown in Table 2. Both methods indicate some residual dextran after dextranase hydrolysis, and, Roberts', some dextran removal in filtration. This is not likely since no DE was used, only a course filter

paper, and it is possibly due to changes in dextran conformation, solubility characteristics or other physico-chemical properties of the dextran molecules, from freezing and melting of the the sugar liquor (after filtration) prior to Roberts' analysis. Reduction of turbidity as a consequence of freezing and melting sugar juices and liquors has been noted before and may be related to the present observation regarding dextran analysis.

Table 1. Raw sugar analysis, ppm dextran

Raw Sugar	SucroTest™	DASA	Roberts'	Haze
1	243	1913	361	139
2	289	2192	462	209
3	101	1134	280	69
4	562	1968	618	439
5	93	1724	225	64
6	101	2021	125	54
7	322	668	453	239
8	154	1879	278	114
9	36	1945	318	49
10	263	0	532	214
11	217	2339	526	194
12	331	2255	249	199
13	86	1413	204	64
14	239	408	380	119
15	254	1266	390	114
16	104	1237	308	134
17	431	1424	585	304
18	90	1111	252	59
19	65	1221	234	49
20	431	1363	503	264

As with the raw sugar samples, it was of interest to test the effect of filtration (with 4 g/200 ml of DE as per the DASA recommendations) on dextran analysis of raw juice samples. SucroTest™ gave consistent results (Figure 2) indicating no dextran removal in DE filtration, while Roberts' again gave lower results on filtered samples when the spurious (high) data point is disregarded, although in this case both filtered and non-filtered samples were kept frozen prior to a during delivery from ASI to SPRI. This "reduction" in dextran is even larger when only points below 1000 ppm are considered. A respectable correlation coefficient R^2 of 0.8142 obtains between Roberts' and SucroTest™ when the high point is disregarded. DASA correlates reasonably well with (filtered) Roberts' and even with SucroTest™ when, again, the high point is disregarded, although giving roughly twice the dextran concentration than either of the other two methods.

Table 2. Raw sugar analysis, after filtration and dextranase treatment, ppm dextran.

Raw Sugar	SucroTest™ after dextranase	SucroTest™ % original	Roberts' after filtration	Roberts' after filtration % original	Roberts' after dextranase	Roberts' after dextranase % after filtration
1	17	7	228	63		
2	23	8	302	65		
3	48	48	184	66	185	101
4	0	0	435	70		
5	25	27	153	68	151	99
6	10	10				
7	44	14			179	
8	170	110	186	67	207	111
9	16	44	217	68	94	43
10	21	8				
11	29	13	245	47	195	80
12	64	19			33	
13	16	19	160	78	223	139
14	77	32	300	79	136	45
15	39	15	183	47	110	60
16	17	16	301	98	86	29
17	21	5	375	64	116	31
18	4	4	132	52	114	86
19	48	74	158	68	136	86
20	37	9	288	57	98	34

For more insight into the DASA polarimetric procedure, a limited repeatability and sensitivity study was performed. Of particular interest are blank tests, illustrated (Table 3) on six 33 Bx raw sugar liquors analyzed as per the DASA instructions, with the exception that the dextranase-carrying filter paper was removed from the vials prior to the analysis. In this case, of course, one expects no change in actual pol reading, and its apparent increase (Control – Test in Table 3) corresponds to an empirical correction built into the software to compensate for the dilution of the sugar liquor (pol reduction) by the solvent (glycerin) released from the dextranase-carrying filter paper. This correction is quite large with respect to the “real” pol change from dextran hydrolysis (Table 4), and even a small relative error in its magnitude will preclude quantitative analysis of dextran. The observation that the DASA procedure appears more robust for cane juice may be related to the fact that this correction is proportional to the pol reading of the measured sample which for the juice samples is 1/3 that of the 33 Bx raw sugar liquors, while the dextran content is generally higher in juice than in 33 Bx raw sugar liquors, making the procedure less vulnerable to the random errors of this internal correction.

Table 3. Optical rotation readings (degrees Z) of six raw sugar samples (33 Bx solutions) in "blank" tests of DASA procedure.

Raw Sugar	Control Reading	Test Reading	Control - Test
A	145.61	146.40	0.79
B	146.16	146.87	0.71
C	143.78	144.55	0.77
D	143.10	143.85	0.75
E	144.63	145.40	0.77
F	143.09	143.89	0.80

Table 4. Optical rotation readings (degrees Z) of five raw sugar samples (33 Bx solutions) in DASA tests.

Raw Sugar	Control Reading	Test Reading	Control - Test	Dextran, ppm	$\Delta Z/100$ ppm Dextran
1	143.08	142.79	-0.29	889	-0.033
15	143.56	143.40	-0.16	481	-0.033
6	144.95	144.70	-0.25	768	-0.033
17	143.67	143.49	-0.18	541	-0.033
2	140.70	140.43	-0.27	833	-0.032

CONCLUSIONS

Roberts' test appears affected by pretreatment, or, more likely, by freezing of the 33 Bx raw sugar liquor prior to analysis, and, if "unfiltered", overestimates dextran over Haze and SucroTestTM in raw sugar by 100–300 ppm and by some 25–50% in raw juice. After filtration, on raw juice, it correlates well with DASA and fairly well with SucroTestTM. SucroTestTM on raw sugar correlates very well with Haze, with a near zero-intercept correlation and close to a 45 degree slope, is unaffected by suspended solids and produces consistent results in raw sugar and juice. DASA gave erratic results on raw sugar, very high on most samples. The possibility of partial dextran removal in filtration of, and incomplete dextran hydrolysis in, raw sugar liquors needs further investigation. An internal correction to pol reading to account for sucrose adsorption and/or solvent (glycerin) release from dextranase-holding filter paper is larger than the change in optical rotation from dextran hydrolysis. On juice samples, correlates well with "filtered" Roberts', but gives about twice the dextran level. A lag of 2–3 hours at room temperature in raw cane juice was observed before dextran concentration began to rise.

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3. V. Singleton, International Sugar Journal, March 2002.

4. AOAC Official Method 988.12 Dextran in Raw Sugar. AOAC Chapter 44, p. 13, 1995.

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Figure 1. Raw sugar analysis. Dextran concentrations in ppm, raw sugar basis.

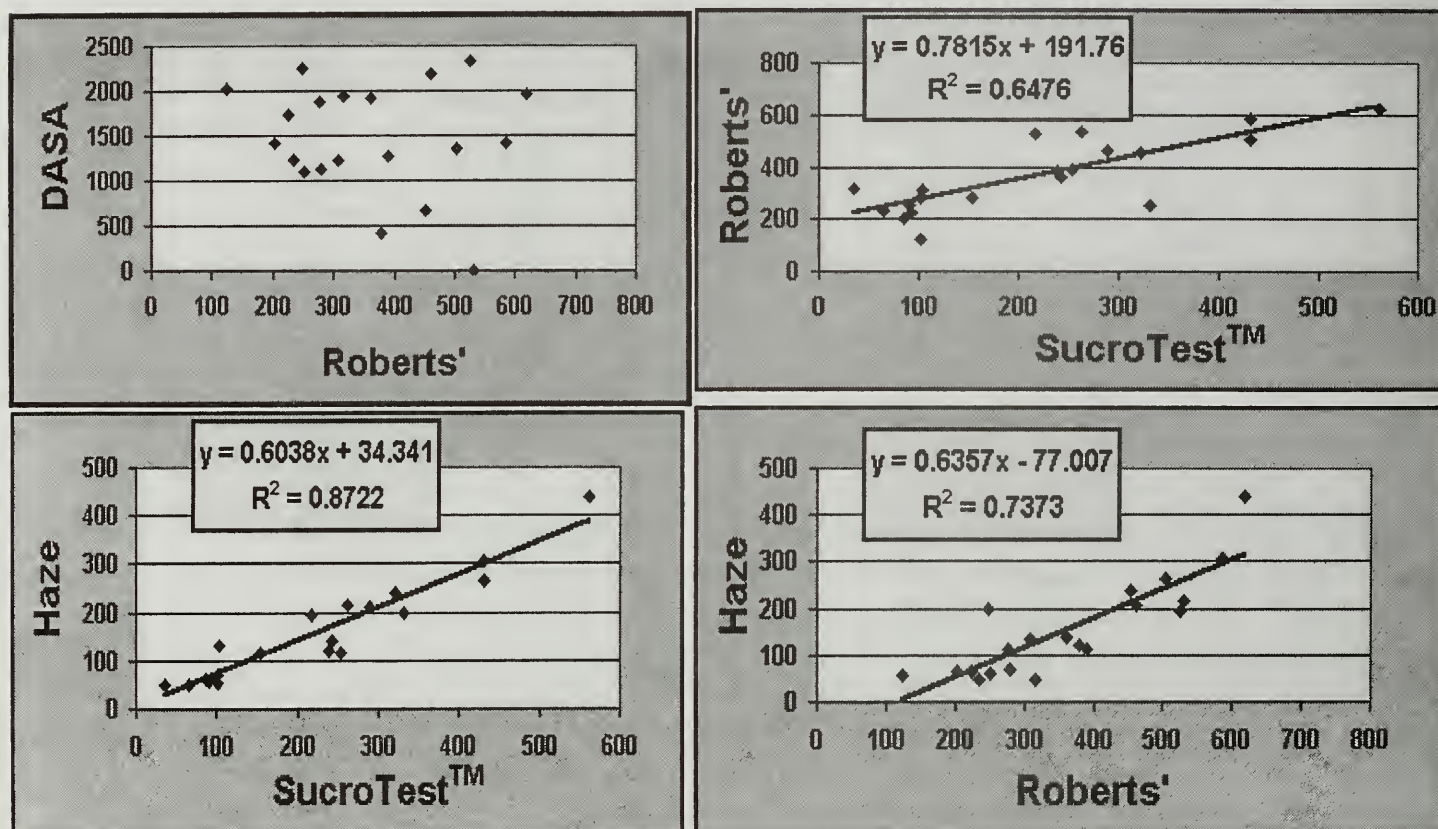
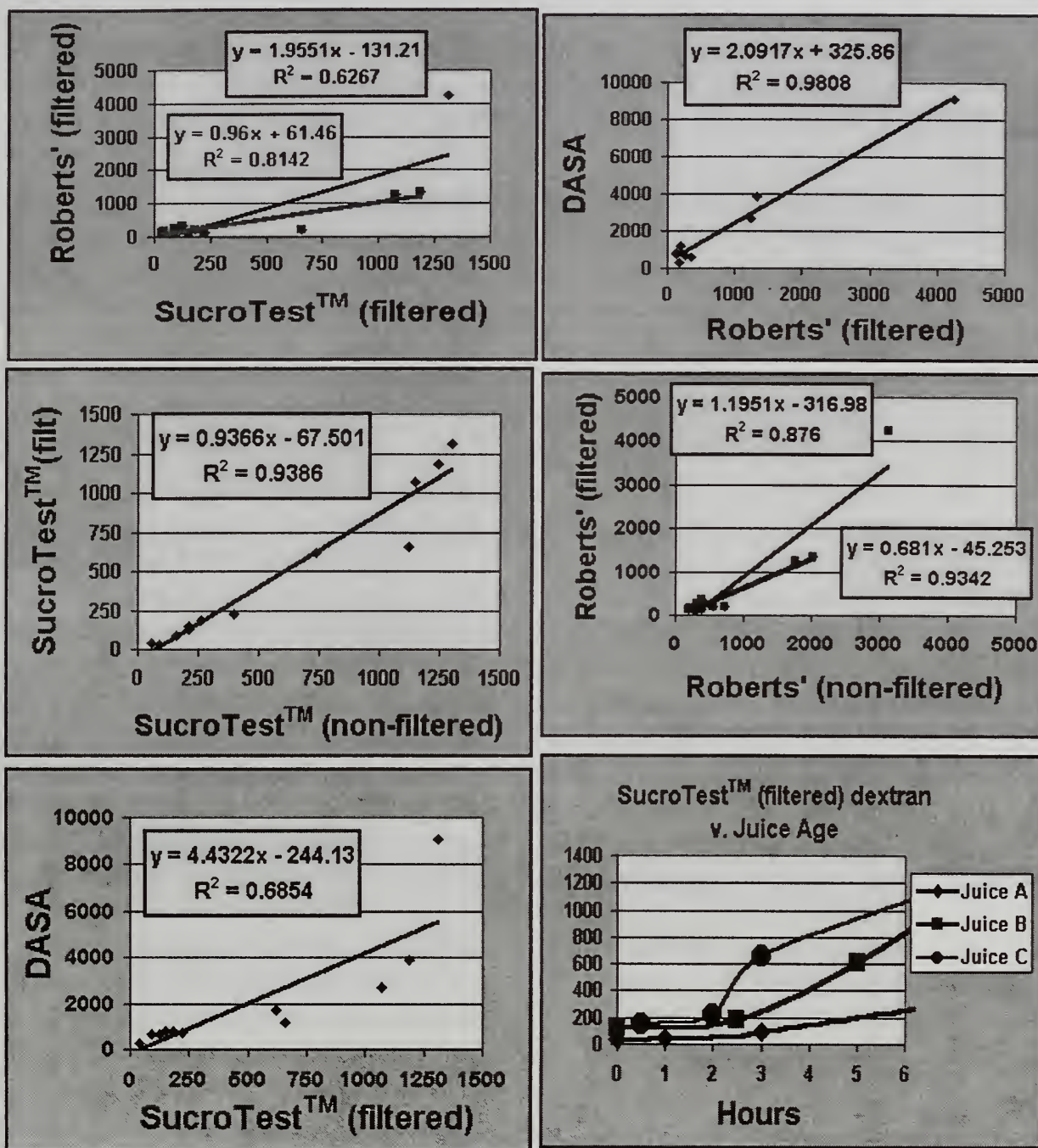


Figure 2: Raw juice analysis. Dextran in ppm on juice (mg/L).



EFFECT OF LOUISIANA SOILS ON CANE JUICE QUALITY

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ABSTRACT

As part of ongoing investigations on the effect of various field practices on the quality of cane juice, we noted that cane juice color decreased significantly when soil was added to assess the effect of soil on cane juice quality. In a study of the 1999 crop in Louisiana, with addition of 5% and 10% soil to the cane juice, it was noted that polysaccharide was also removed, the first time this had been reported. These observations run contrary to expectations that soil will degrade the quality of cane juice. Raw juice from green cane, which had been topped, but still retained side leaves, was treated with 10% added soil. Two soils from the Louisiana cane growing area, Sharkey clay and Norwood silty clay loam were tested. The juice was treated for 30 minutes in a shaker either at room temperature (25° C) or heated (80° C). Changes in pH, color, total polysaccharide, ash and filtration rate were noted. Both soils decreased color and total polysaccharide and increased the filtration rate. pH and ash were not significantly changed, nor was the purity.

INTRODUCTION

It is generally accepted that “trash” (combinations of field soil, or mud, and leaves, dead and/or green) will degrade the quality of cane juice by increasing the color and decreasing the purity (Fors and Arias, 1997, Purchase, *et al.*, 1991, Ivin and Doyle, 1989, Godshall, *et al.*, 2000). While this is certainly true, in experiments to test the effect of individual constituents of trash, we noted that soil alone actually decreased the color and polysaccharide concentration in juice, speeded up filtration and did not seem to have an adverse effect on ash, pH or purity.

Louisiana Soils. Sugarcane is grown mainly in the soil areas known as the Subtropical Mississippi Valley Alluvium, with the dominant soils being Sharkey, Mhoon and Commerce. Some cane is also grown in the extreme southern part of the Red River Valley in Norwood Soil. Table 1 shows some of the properties of the soils in which cane is grown.

Table 1. Properties of the soils in Louisiana in which cane is grown.

Soil	Type	CEC*	% Clay	% Sand
Norwood	Reddish, silty loam soil	9.4	13.6	46.8
Commerce	Silty loam	11.8	16.3	36.4
Mhoon	Grey, silty clay loam	n/a	n/a	n/a
Sharkey	Clay	30.5	49.4	26.5

*CEC = Cation Exchange Capacity

The cation exchange capacity (CEC) of a soil is the sum of the basic cations present on the soil matrix. It is used as an index of the total exchange capacity of the soil. It increases in soils as the clay content increases.

MATERIALS AND METHODS

50 ml of raw cane juice from topped cane, with side leaves, was treated with 5 g of Norwood or Sharkey soil and placed on a gyratory shaker for 30 min. Experiments were conducted at 25°C and 80°C. Treated juice was analyzed for pH, color, total polysaccharides, ash, and filtration rate. Color and conductivity ash were measured using standard ICUMSA methods. Total polysaccharides were measured by the SPRI method. Filtration rate was determined as ml cane juice that passed through a 0.45 μ m membrane in 5 min, reported as ml/min.

The Sharkey and Norwood soils were settled in water to remove leafy material and allowed to air dry.

In earlier experiments, cane juice obtained in various harvesting regimes were tested: topped and stripped, topped with side leaves, with tops and side leaves, topped with side leaves plus 5% Sharkey, and topped with side leaves plus 10% Sharkey. Six replicates of each treatment were analyzed.

RESULTS AND DISCUSSION

Figure 1 shows the effect of Mhoon soil on cane juice color from an earlier experiment, previously reported (Legendre, *et al.*, 1996). It shows that the soil removed a small amount of color but that leaves contributed much more color to the juice. When mud and leaves were combined, the competing effects of each were evident.

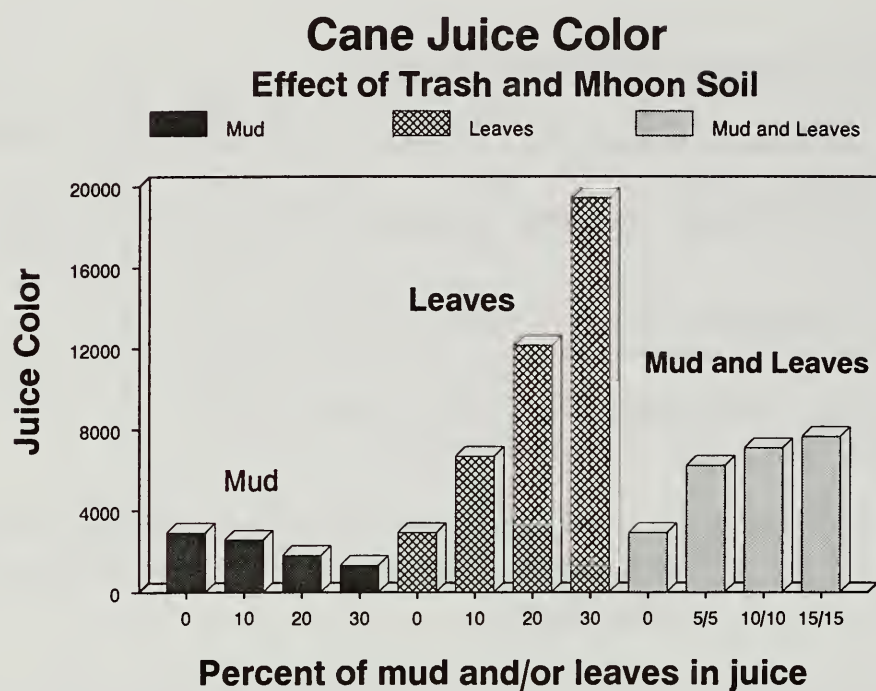


Figure 1. Effect of Mhoon soil and green leaves on cane juice color (from Legendre, *et al.*, 1996).

Figure 2 shows the effect of Sharkey soil on color. Sharkey had little effect on the color, but did help to mitigate the effect of leaves in that less color was added to the juice in the presence of a combination of leaves and soil.

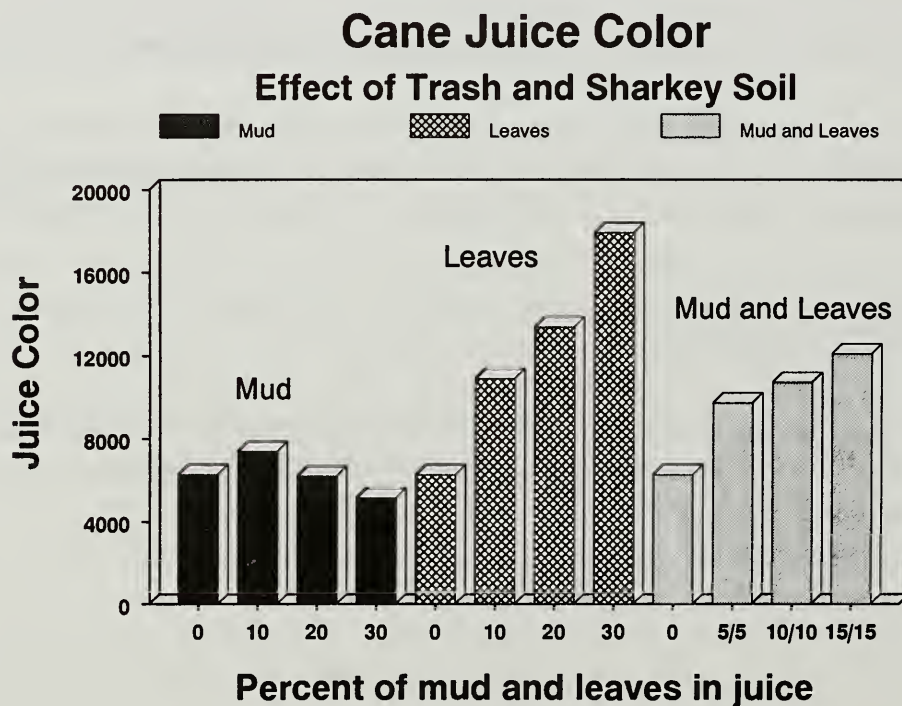


Figure 2. Effect of combinations of Sharkey soil and trash on cane juice color.

Figures 3 and 4 show the effect of various treatments on cane juice color and polysaccharides. Addition of 5% Sharkey to the control juice (topped but with side leaves) decreased color and polysaccharides almost to the level of hand harvested clean cane juice (first bar, topped and stripped cane). This represents a decrease of 20% color and 30% polysaccharide.

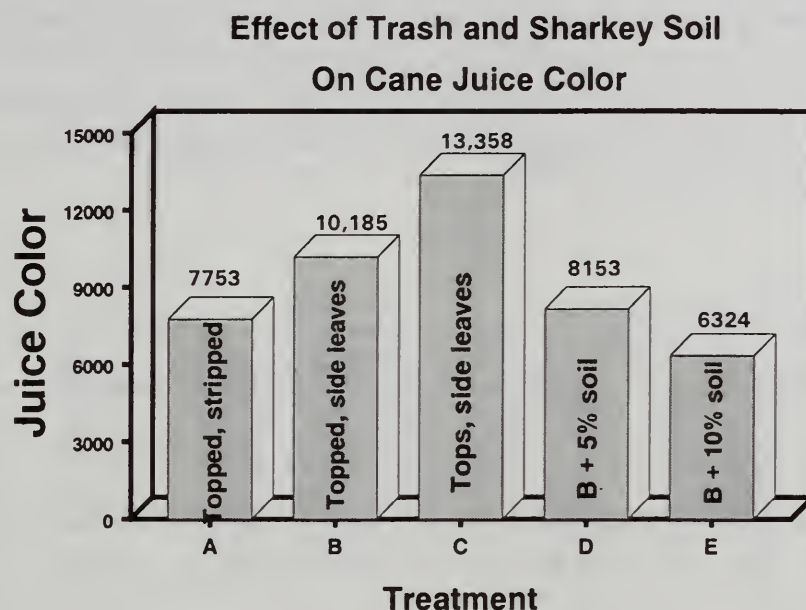


Figure 3. Effect of trash and Sharkey soil on cane juice color.

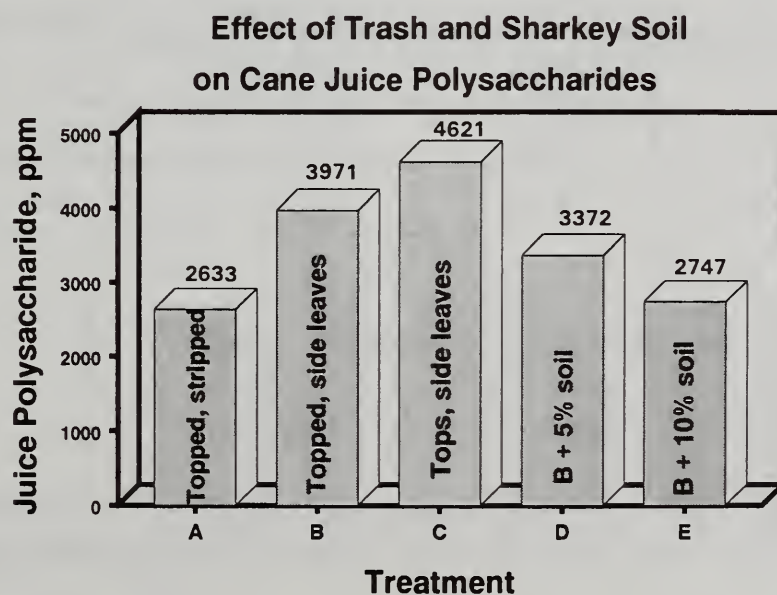


Figure 4. Effect of trash and Sharkey soil on cane juice polysaccharide concentration.

Effect of Heat on Cane Juice. Table 2 shows the effect of heat (80°C for 30 min) on cane juice. Heat decreased color 4.33% and polysaccharide 6.05%. Ash increased 4.69% and filtration rate improved 14.9%. There was no change in pH. The increase in ash may represent the solubilization of inorganic matter present in the juice caused by the heating. (However, a change in ash from 2.56% to 2.68% is not considered significant.)

Table 3 shows the effect of Norwood on raw juice at 80°C for 30 min. There was little effect on color or pH, compared to the control (Table 2). Ash decreased a small amount (0.75%), but it is a significant change from the 4.69% increase in the heated juice. Polysaccharides decreased significantly (21.1%) and filtration improved 20% over the improvement caused by heat alone. Each experiment was repeated six times.

Table 2. Effect of heat on raw cane juice.

Sample	pH	Color	Total Polys	Ash	Filtration
25° C	5.62	10,129	5277	2.56	0.87
80° C	5.59	9,690	4958	2.68	1.0
% Change	-0.53%	-4.33%	-6.05%	+4.69	0.149

Table 3. Effect of Norwood soil on raw cane juice at 80° C.

Sample	pH	Color	Total Polys	Ash	Filtration
Control	5.59	9960	4958	2.68	1/0
Norwood	5.62	9428	3911	2.66	1.35
% Change	+0.54%	-5.3%	-21.1%	-0.75%	0.35

Summary of results with treatment of Sharkey and Norwood soils.

pH. No significant change with either soil at either temperature.

Color. Sharkey removed 14.1% color at 25°C, but only 5.2% at 80°C. Norwood removed 5.0% at 25°C and 2.7% at 80°C. These results indicate a release of color at higher temperatures.

Polysaccharides. Both soils removed significant amounts of polysaccharides. Sharkey clay removed 24.6% polysaccharides at 25°C and 17.7% at 80°C. These results are similar to those previously encountered with the Sharkey clay (Figure 4.) Norwood removed 18.6% at 25°C and 21.1% at 80°C. The effect on polysaccharides is shown in Figure 5 (Norwood) and Figure 6 (Sharkey).

Ash. Sharkey had a 4-5% decrease in ash. Norwood caused a small increase of ash, 2.73% at 25 °C and a slight decrease of 0.75% at 80 °C.

Filtration rate. Norwood increased the filtration rate 26.4% at 25 °C and 35.0% at 80 °C. Sharkey clay doubled the filtration rate at 25 °C, but showed no change at 80 °C. This result may be anomalous, as several previous filtrations with a different batch of Sharkey clay in cane juice had shown as much as a 10-fold increase in filtration rate at room temperature.

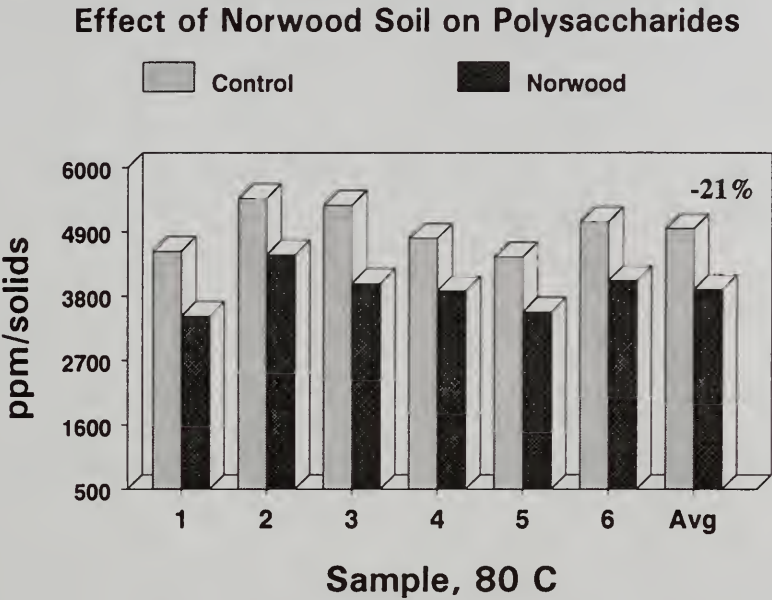


Figure 5. Effect of adding 10% Norwood soil to cane juice on polysaccharide content.

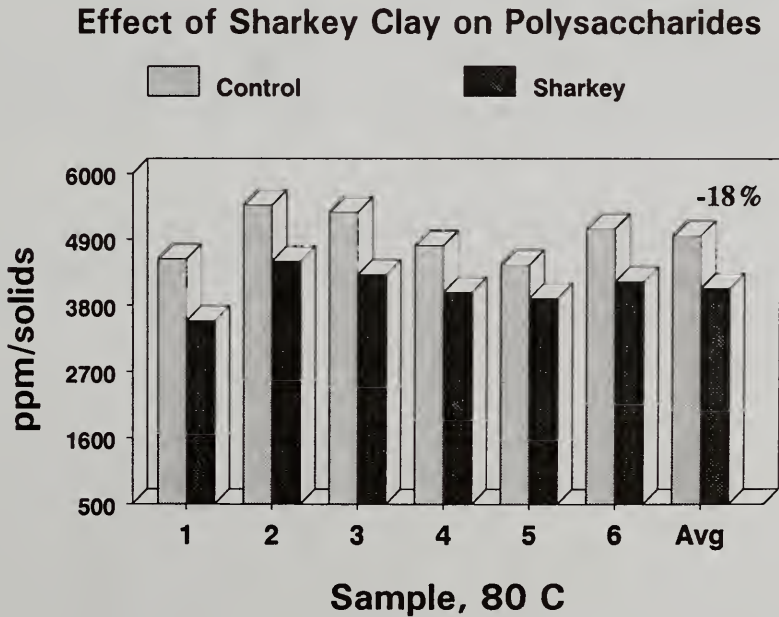


Figure 5. Effect of adding 10% Sharkey soil to cane juice on polysaccharide content.

CONCLUSIONS

These studies show that the soils in the cane growing areas of Louisiana, Mhoon, Sharkey and Norwood, have the ability to remove significant amounts polysaccharide from cane juice. There were no deleterious effects on pH or ash, and varying amounts of color were removed. The presence of these soils can reduce the amount of color and polysaccharides imparted by leaves to the juice. Filtration rate is sometimes also improved.

This work is not intended to advocate or recommend bringing soil in with harvested cane. The cleaner the juice, the better in the long run. Soil has destructive effects on the mills, increases the burden on the clarifier and contributes to disposal costs. The results are of considerable interest, however, because they can help explain some anomalous behavior in cane juice quality when a lot of mud is brought into the mill. It may be possible, in the future, to consider how to exploit the beneficial effects of the soils in the cane growing area of Louisiana, while avoiding their negative effects.

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